# CLINICAL PATHOLOGY
AND
# PRACTICAL MORBID HISTOLOGY

T. STRANGEWAYS PIGG

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# CLINICAL PATHOLOGY

AND

# PRACTICAL MORBID HISTOLOGY

BY

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OF CAMBRIDGE.

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# PREFACE.

IN this manual I have aimed at giving a few reliable methods by means of which the student may be enabled to make any of the ordinary pathological examinations, and at the same time acquire a sound knowledge of technic. The illustrations are diagrams only.

TO THE STUDENT.—The student is urged to follow step by step the directions given, and in a short time he will be able to work rapidly, neatly, and well. It cannot be put too strongly before him that a mere acquaintance with a number of methods will never make him a good worker. Good technic alone makes a method useful, and a valuable method is often rejected as useless owing to bad technic. This suggests the reason why some will allow only those methods to be of use with which they are familiar, and which experience has taught them how to use. An instance is the use of Jenner's stain for blood, which many have pronounced unsatisfactory. The stain is one of the most reliable and useful we have, and at the same time the simplest of all blood stains—so simple that a failure practically never occurs.

T. S. P.

*Cambridge, June 1901.*

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# DESCRIPTION OF PLATES.

WET blood films, from which the following drawings are made, show more structure than can be seen in dry films. Stained by Jenner's eosine-methylene blue in absolute methyl alcohol ; drawn with Abbe camera lucida, Zeiss oc. 4 and Leitz obj. $\frac{1}{12}$ oil immersion. × 1000.

## PLATE $I^{A}$.

**Figs. 1, 2, & 3. Finely granular eosinophile leucocytes (polynuclear or neutrophile leucocytes of Ehrlich).**

Extremely irregularly shaped nucleus, with very deeply staining network and large masses of chromatin. Very fine granules, staining pink with eosine, but less intensely so than in the following.

**Figs. 4, 5, & 6. Coarsely granular eosinophile leucocytes (eosinophile leucocytes of Ehrlich).**

The nucleus, composed of two or three separate rounded lobes connected by a slender filament, or horseshoe or S-shaped, has a delicate paler network and smaller collections of chromatin than the nucleus of the finely granular eosinophile leucocytes.

Large and some smaller round granules, staining in pure colour of eosine ; some are more deeply stained at their periphery.

**Figs. 7, 8, 9, 10, & 11. Lymphocytes.**

Small nucleus—usually round, sometimes oval, a few kidney-shaped—with deeply staining network and collections of chromatin.

Protoplasm pale and transparent.

In Fig. 10 the cell is spread out in a drying edge of the film.

**Figs. 12 & 13. Hyaline leucocytes,** smaller size.

**Figs. 14, 15, & 16. Hyaline leucocytes,** large size **(large mononuclear leucocytes of Ehrlich).**

Large nucleus—usually kidney-shaped or showing three bulgings (often appears round in dry films), seldom round—with slightly marked paler network and fewer collections of chromatin than in the lymphocytes, while the protoplasm has the appearance of ground glass and is less transparent.

**Figs. 17 & 18. Finely granular basophile leucocytes.**

The nucleus is usually trilobed when not obscured by granules, with a very pale delicate network. The granules are very irregular in size, and also in shape, and stain metachromatically an intense violet-blue colour.

**Fig. 19. Red blood corpuscles,** showing normal colour and variation in size.

## PLATE I$^B$.

**Figs. 20, 21, & 22. Finely granular eosinophile myelocytes (myelocytes of Ehrlich).** From blood film of myelogenic leucæmia.

Large round or kidney-shaped nucleus—often eccentrically situated —with pale scattered patches of chromatin, but ill-defined network and no line of chromatin defining the outline.

Very fine violet granules, corresponding in staining with those of the finely granular eosinophile leucocytes, but distinctly less oxyphile.

These cells are normally situated in the bone marrow, and form the source of the finely granular eosinophile leucocytes of the blood.

**Fig. 23. Coarsely granular eosinophile myelocyte (eosinophile myelocyte of Ehrlich).** From blood film of myelogenic leucæmia.

The nucleus has the same characters as in Fig. 20, but its outline is obscured by the large round granules, which stain pink with a bluish tinge, corresponding in staining with those of the coarsely granular eosinophile leucocytes, but being distinctly less oxyphile. These cells are normally situated in the bone marrow, and form the source of the coarsely granular eosinophile leucocytes of the blood.

**Fig. 24 Hyaline cell.** From blood film of myelogenic leucæmia.

Round or oval nucleus, with same characters as in Fig. 20, and even less chromatin, surrounded by hyaline protoplasm resembling ground glass. This cell occurs normally in the bone marrow.

**Fig. 25. Coarsely granular basophile cell ('mast zellen' of Ehrlich).** From blood film of myelogenic leucæmia.

The nucleus has the same characters as in Fig. 20, but its outline is obscured by very large and some smaller round granules, characterised by their metachromatic staining, some being intense violet-blue, and others a lighter violet colour.

This cell appears in the blood in myelogenic leucæmia, though infrequently.

**Fig. 26. Small very finely granular basophile cell.** From blood film of myelogenic leucæmia.

This is a common form of basophile cell in the blood of myelogenic leucæmia.

**Figs. 27, 28, 29, 30, & 31. Normoblasts.** Figs. 27, 28, and 30 are from blood films of myelogenic leucæmia, Fig. 29 is from a blood film of pernicious anæmia.

Figs. 27 and 28 are typical. Size, shape, and colour of normal red blood corpuscles, but containing a round intensely stained nucleus, with its contour sharply defined by a dark rim, and in which but little structure, or darker bars and patches, can be distinguished. Fig. 27 shows a clear space round the nucleus. Fig. 29 is large, but the nucleus is characteristic. Fig. 30 shows a lobed nucleus which may divide into several separate nuclei. In Fig. 31 the protoplasm shows polychromatophilia.

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**Fig. 32. Normoblast nucleus,** with only **trace of protoplasm.** (? microblast.) From blood film of pernicious anæmia.

**Fig. 33. Free normoblast nucleus.** Easily distinguished from a lymphocyte with no visible protoplasm by characters described above.

In dry films from blood of pernicious anæmia blue dots are sometimes seen scattered throughout the protoplasm of a few normoblasts and red corpuscles.

Normoblasts are normally found in adult bone marrow as the precursors of the red blood corpuscles.

**Figs. 34, 35, 36, 37, 38, & 39. Megaloblasts.** From blood films of pernicious anæmia.

Figs. 34, 38, and 39 are typical. Two to three times as large as normal red blood corpuscles or normoblasts.

The nucleus—often eccentrically situated—is rounded or irregularly pear-shaped; it is lightly stained; its outline is defined only by the surrounding protoplasm or by a fine pale chromatin line, and it contains a pale delicate network and patches of chromatin.

The protoplasm very often shows polychromatophilia in various shades of violet, and is frequently separated from the nucleus by a clear space.

Fig. 37, megaloblast with a lobed nucleus.

Compare Figs. 35 and 36, which are smaller and shrunken megaloblasts, with Fig. 29, a large normoblast, and Fig. 31, a normoblas showing polychromatophilia in its protoplasm.

Megaloblasts are normally found in fœtal bone marrow, and are embryonic forms of nucleated red corpuscles.

**Fig. 40. Gigantoblast.** From blood film of pernicious anæmia.

Is simply an unusually large megaloblast. Occurs seldom in blood.

**Fig. 41. Megaloblast showing karyokinesis.** From blood film of pernicious anæmia.

Occurs seldom in blood. Karyokinesis is occasionally seen in normoblasts, especially in leucæmia.

**Fig. 42.** Red blood corpuscles showing **poikilocytosis. Polychromatophilia** is shown by two corpuscles in this group, and by the protoplasm of all the megaloblasts drawn, fig. 35 showing the deepest violet discoloration.

---

N.B.—For a standard to compare the size of cells, the normal red blood corpuscles of Fig. 19 must be taken, and not the three abnormally large red blood corpuscles (megalocytes) in Fig. 42.

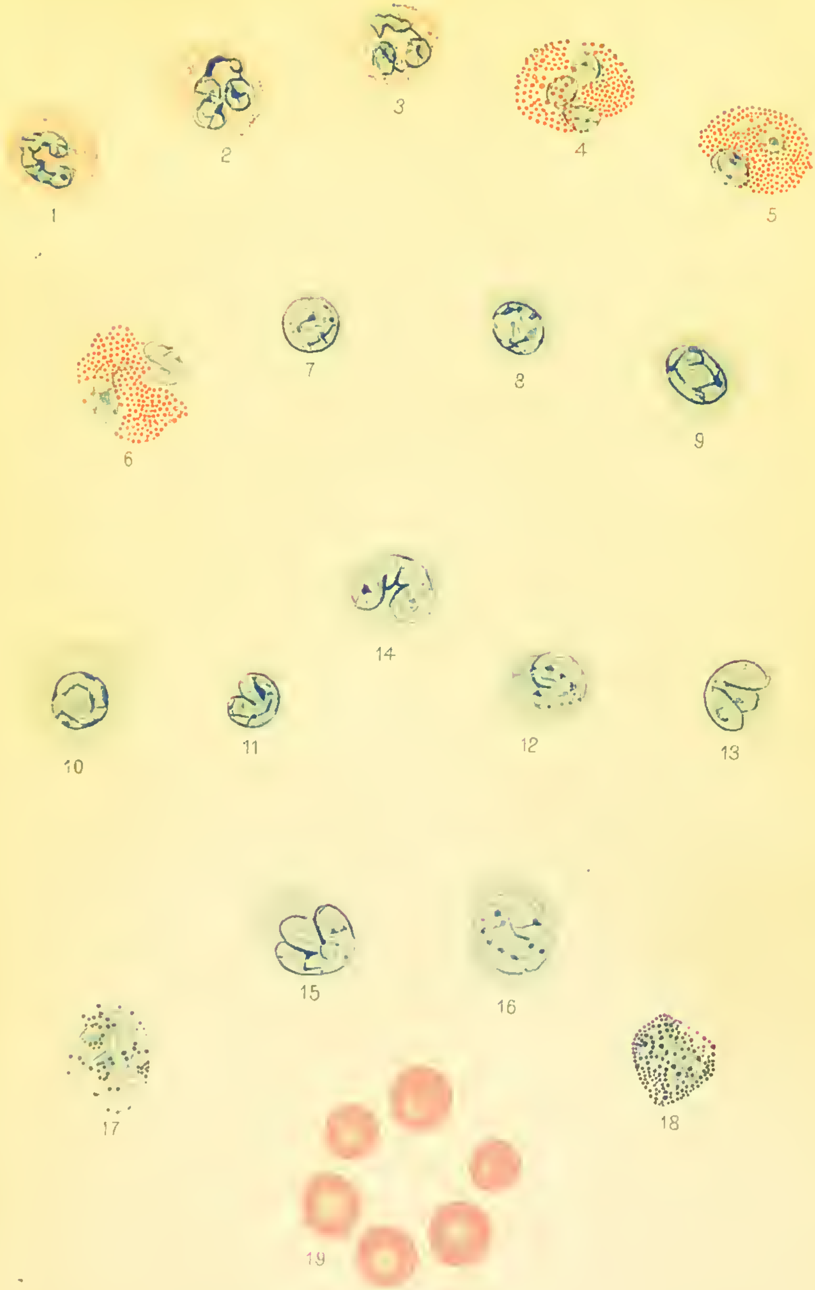
G. Scott.

## DESCRIPTION OF FORMALIN VAPOUR ABSOLUTE ALCOHOL—WET METHOD USED FOR THE BLOOD FILMS.

1. Hold the wet film immediately after making it—wet side down—in the mouth of a wide bottle half filled with undiluted 40 per cent. formalin for not more than *fiveseconds*.
2. Drop, *while still wet*, film downwards, into absolute alcohol. Leave an hour, or better one to two days (to get rid of the formalin, so that the red corpuscles stain red; this can be done more quickly by placing specimens, not covering each other, film upwards in a capsule containing alcohol).
3. Blot off excess of alcohol.
4. Immediately, before any drying occurs, drop on a few drops of Jenner's eosine-methylene blue stain with a dropping pipette; cover with a watch glass to prevent evaporation. Stain for *two minutes*—not any longer.
5. Allow excess of stain to run off, and rinse at once in a bowl of *distilled* water, and then in fresh distilled water for one to two minutes, till the film is a red-violet colour.
6. Blot off excess of water.
7. Dehydrate very rapidly in absolute alcohol, merely dipping in and out as quickly as possible.
8. Immediately wash off alcohol in first xylol, wash in second xylol, drop on fresh xylol.
9. Mount in xylol balsam.

N.B.—The film must not be allowed to dry at *any* stage; dehydrate very quickly in the absolute alcohol; use only pure distilled water (or filtered rain water); use cover-glasses which are free from acid or alkali. ; *Journ. of Path. and Bact.*, Dec. 1900.

Plate IA



G Scott del

E Wilson lith

NORMAL CELLS OF BLOOD
From preparations by the Hon G Scott

Clinical Pathology
Strangeways · Pl. II

Plate IB

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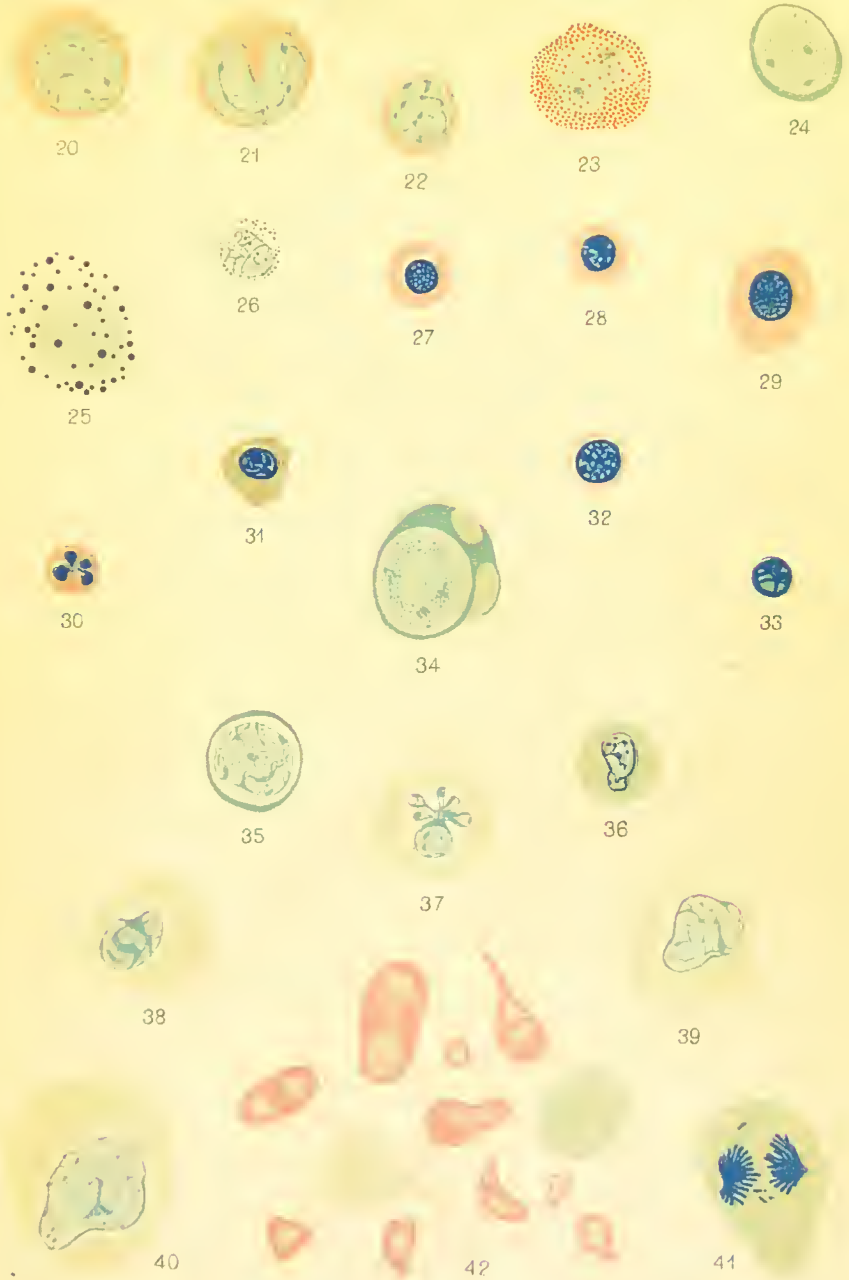

G. Scott del. E. Wilson lith.

ABNORMAL CELLS APPEARING IN THE BLOOD
From preparations by the Hon. G. Scott

# CLINICAL PATHOLOGY.

## BLOOD.

### I.—TO CLEAN APPARATUS USED IN EXAMINATION OF BLOOD.

Apparatus, &c.—Rubber bellows, porcelain evaporating dish, glass rod, forceps, tripod, sand-bath, small glass capsules, distilled water, absolute alcohol, ether, 10 % solution of caustic potash, solution of potassium bichromate, and sulphuric acid (ix.), fine brass gauze.

#### A.—To Clean Cover Slips and Slides.

1. Boil them in the bichromate solution for 1 hour in the evaporating dish on a sand-bath, adding fresh solution from time to time as it evaporates.
2. Wash repeatedly with distilled water.
3. Keep in absolute alcohol in a wide-mouthed stoppered bottle.
4. Just before use, burn them over a Bunsen flame on a clean piece of fine brass gauze.

Note.—*If they are to be used in the preparation of blood films, dry them with a soft linen rag, do not burn them.*

#### B.—To Clean Thoma-Zeiss Pipettes.

1. With the bellows blow from the pipette any liquid remaining in it.
2. Draw up some distilled water and rotate the pipette.
3. With the bellows blow out the water from the pipette.
4. Repeat this several times.
5. Draw up some absolute alcohol and rotate as before.

6. Blow out the alcohol as before.
7. Repeat 5 and 6.
8. Fill the pipette with ether and rotate.
9. Blow out the ether.
10. Repeat 8 and 9.

NOTE.—(*a*) *If the pipette is quite clean the glass bead in the bulb will revolve freely when the pipette is rotated; if the bead adheres to the sides of the bulb the whole process must be repeated.*

(*b*) *If the blood should clot in the pipette, place the pipette in a solution of caustic potash till the clot is dissolved. Never place caustic potash in the counting chamber.*

### C.—TO CLEAN THE CAPILLARY PIPETTE OF THE VON FLEISCHL'S HÆMOMETER.

Proceed as above, using a pipette with a fine point to blow the various liquids through the capillary pipette.

NOTE.—*Always clean the cover slips and pipettes yourself; do not trust to other hands.*

## 2.—METHOD OF OBTAINING THE BLOOD.

APPARATUS, &c.—Sharp surgical needle, clean handkerchief, spirit lamp, clean cover slips and slides.

1. Sterilise the needle in the flame of a spirit lamp.
2. Take the lobe of the ear between the thumb and forefinger of the left hand, and turn its posterior aspect forward.
3. Make a sharp and fairly deep puncture with the needle on the back of the lobe.

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4. Wipe away the first drop of blood with a clean handkerchief.

NOTE.—*The blood will usually flow freely and no pressure need be applied, but in cases of severe anæmia slight pressure may be required: this should always be applied as far as possible from the puncture.*

*The blood may also be taken from a finger, by winding around it a piece of tape or a rubber band, and then giving a sharp prick below the nail: in this case also avoid pressure.*

## 3.—MICROSCOPIC EXAMINATION OF FRESH BLOOD.

APPARATUS, &c.—Clean cover slips (I. A), glass slides, and forceps, in addition to that required for (2).

1. Prepare carefully clean cover slips and slides. (1.)
2. Obtain a drop of blood. (2.)
3. Take up a clean cover slip and with it touch the summit of the drop of blood without touching the skin.
4. Quickly place the cover slip face downwards upon a clean glass slide; the blood will at once spread out as a thin even film under the glass slip.
5. Examine under a high power without the condenser, using the concave mirror and diaphragm.

NOTE.— *a) The following points can be made out:—*

(1) *Rouleaux formation of the red blood corpuscles.*
(2) *Alteration in the shape and size of red blood corpuscles. Notice any deformity.*
(3) *Alteration in relative number of white blood corpuscles to red.*
(4) *Decrease of hæmoglobin if marked.*
(5) *The amount of fibrin present.*
(6) *Any increase in the number of 'blood plates.'*

(*b*) *The following parasites when present can be studied with advantage by this method:—Plasmodium of malaria, spirochæte of relapsing fever, filaria sanguinis hominis.*

## 4 —ESTIMATION OF HÆMOGLOBIN BY MEANS OF VON FLEISCHL'S HÆMOMETER (Fig. 1).

APPARATUS, &c.—Von Fleischl's hæmometer, surgical needle, distilled water, candle, fine pipette.

1. Place the frame carrying the wedge-shaped piece of coloured glass (Fig. 1, A) into its position on the stage of the instrument (Fig. 1, B).

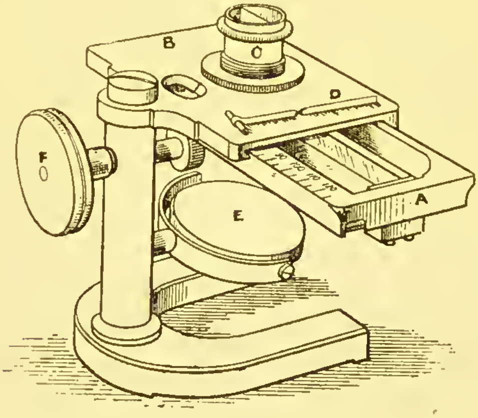

Fig. 1.

A, Coloured glass; B, Stage; C, Mixing-cell; D, Capillary pipette; E, Reflector; F, Milled screw-head.

2. Take the mixing-cell (Fig. 1, C) with one chamber quarter full of distilled water, and place it in slot of stage.
3. Take a pipette with a fine end, and fill it with distilled water. *Call this pipette* A.
4. Take the capillary pipette supplied with the apparatus (Fig. 1, D), *it must be scrupulously clean*, and, having obtained a drop of blood in the usual way (2), place one end of the pipette at the edge of the drop, and the blood will at once fill the pipette.

5. Take the pipette A, lying ready, and containing distilled water, place it to the end of the small collecting pipette and blow out the blood into the mixing-cell containing the distilled water. This will give a mixture of blood and distilled water in one chamber of the mixing-cell.

6. With the pipette A, fill completely both chambers of the mixing-cell, being careful to mix thoroughly the blood in the cell which contains the diluted liquid. When this is done, one chamber of the mixing-cell should be full of diluted blood, the other full of distilled water without any blood in it. The mixing-cell must be full to the brim in both chambers, but neither must overflow into the other.

7. Adjust the mixing-cell so that the compartment containing the distilled water comes over the slip of coloured glass, the light being reflected through this, whilst the compartment containing the diluted blood receives the light direct from the reflector (Fig. 1, E).

8. To read off the amount of hæmoglobin :—
    (*a*) Do not stand or sit facing the light, but sideways. (*This is important.*)

    (*b*) Use as little light as possible (*always from a candle*). Roll up a piece of black paper into a tube, and look through this.

    (*c*) Use first one eye and then the other, and never look for more than a few seconds at a time.

    (*d*) Move the milled screw-head (Fig. 1, F) with short, quick turns, rather than slowly and gradually, until the tint of the glass wedge exactly corresponds to the tint of the diluted blood.

(*e*) The reading on the scale will give the percentage of hæmoglobin.

Note.—*If the Von Fleischl hæmometer is not to be obtained the estimation of the hæmoglobin from the specific gravity of the blood is a very useful substitute, except in cases where there is œdema or dropsy.*

## 5.—ESTIMATION OF RED AND WHITE BLOOD CORPUSCLES WITH THOMA-ZEISS APPARATUS (Fig. 2).

Apparatus, &c.—Thoma-Zeiss apparatus, Toison's fluid (xlviii.), watch-glasses, surgical needle, glacial acetic acid solution (xxvi.).

### A.—Collecting and Diluting the Blood.

1. Be sure the pipettes (Fig. 2, A and B) are absolutely clean. (1.)

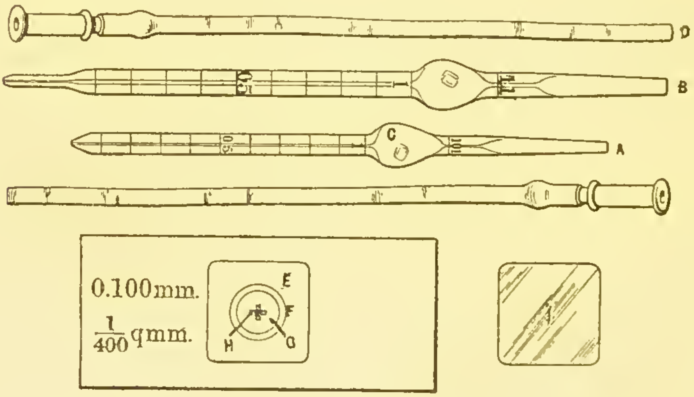

Fig. 2.

A, 'Red counter'; B, 'White counter'; C, Glass bead; D, Rubber tube; E, Counting chamber; F, Moat; G, Disc; H, Ruled squares; I, Cover glass.

2. Filter into a watch-glass a little Toison's fluid.
3. Puncture the lobe of the patient's ear and obtain a large drop of blood. (2.)

4. Place the point of the red counter (Fig. 2, A) into the drop of blood, and gently draw up the blood to the mark 0·5 on the pipette; the blood must reach exactly to the mark, and not beyond it.
5. Quickly place the point of the pipette into the Toison's fluid, and draw up the solution so that it reaches the mark 101 on the pipette; it is of the utmost importance that the fluid should be drawn up exactly to this mark and not beyond; if the fluid should pass the mark, the whole process must be repeated, previously cleaning the pipette.
6. Mix the blood and the diluting solution thoroughly by rotating the pipette for 1 minute in such a manner that the glass bead (Fig. 2, C) revolves freely in the bulb. (*The blood is now ready for examination, but this may be deferred for some hours if necessary.*)
7. Remove the rubber tube (Fig. 2, D), and blow out 5 to 6 drops from the pipette, then wipe the end.
8. Place in the counting chamber (Fig. 2, E) a small drop of the diluted blood of such size that when the cover glass is placed on the chamber, the disc (Fig. 2, G) is covered, or nearly so, but none of the diluted blood is spilled over into the moat (Fig. 2, F) around the chamber; *this direction must be carefully carried out.*
9. Press the cover glass (Fig. 2, I) firmly down upon the slide, so that 'Newton's rings' can be clearly seen.
10. Stand it aside for 5 minutes to allow the blood corpuscles to settle; *the diluted blood is now ready for examination.*

### *To collect Blood for the Estimation of White Corpuscles.*

Take the pipette (Fig. 2, B) graduated 1 in 10, and draw up blood to mark 0·5, then draw up acetic acid solution to mark 11, and proceed exactly as with the 'red counter.'

NOTE.—*This solution renders the red blood corpuscles invisible, but the white corpuscles stand out clearly.*

### B.—COUNTING THE RED BLOOD CORPUSCLES.

1. Use a microscope with Zeiss D lens, concave mirror, and narrow diaphragm, without the condenser.
2. Having focussed the blood corpuscles, find the ruled lines on the floor of the counting chamber (Fig. 2, H).
3. Move the slide slowly over the stage, so that a general view of the whole chamber may be obtained, and notice carefully if the blood corpuscles are fairly evenly distributed over the floor of the counting chamber; if the mixing has been properly carried out, this will be the case.
4. Having satisfactorily determined the positions of the squares, take one of the sixteen large squares, formed by a line ruled through every fifth small square, and fix the slide so that this is in the centre of the field. A Zeiss D will take the whole square in one field (Fig. 3).
5. To count, proceed as follows:—
   Count all the corpuscles lying wholly inside the top left-hand smaller square, and also all those resting upon or impinging on the ruled lines forming the top and left-hand side of the square, whether the corpuscles be inside or outside the smaller square, but do not count those impinging on the bottom or right-hand side of the square, even though they lie inside it.
6. Count, in a similar manner, the corpuscles inside and impinging on the lines of the next square to the right.

7. Count 5 squares to the right, and then count the 5 squares under these, again from left to right as in reading.
8. Repeat this four times, till 20 squares have been counted; it will then be found that all the corpuscles lying wholly inside the rectangle, formed by the 20 squares, have been counted, and also all those impinging on the upper and left-hand side of this rectangle, but none of those impinging on the bottom or right-hand side.

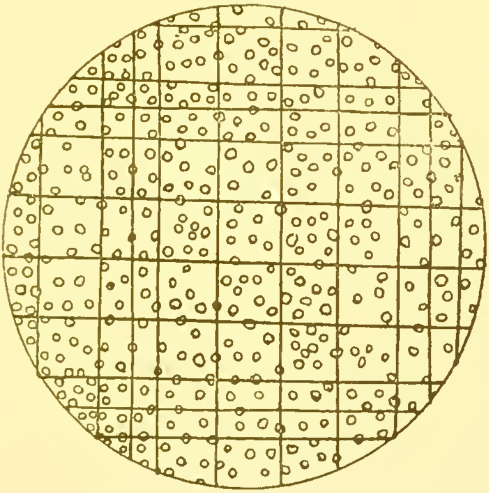

Fig. 3.

9. Make five counts of 20 squares each.
10. To calculate the number of red blood corpuscles per cubic millimetre, proceed as follows:—

$$\text{The No. of corpuscles per c. mm.} = \frac{\text{No. of corpuscles counted} \times \text{No. of times blood was diluted} \times 4000}{\text{No. of squares counted.}}$$

NOTE.—*To estimate the number of white corpuscles with the red counter proceed as above, but count the whole 400 squares several times, refilling the counting chamber for each count. The white corpuscles are stained blue by the Toison's fluid.*

### C.—COUNTING THE WHITE BLOOD CORPUSCLES WITH THE 'WHITE COUNTER.'

1. Proceed as in 1 to 3 in B.
2. Find the top left-hand larger square, and count all the white corpuscles lying within it or impinging on the top and left-hand sides of this larger square. *All the corpuscles lying within 25 small squares will then have been counted.*
3. Move the slide, and bring into the field the next large square to the right, and count as before.
4. Again move the slide from left to right and count.
5. Repeat. *Four larger squares of 25 small squares each will then have been counted.*
6. Move the slide now upwards, and bring into the field the large square below the last counted, and count as before.
7. Move the slide from right to left, and again count.
8. Proceed in a similar way to count all the larger squares until the whole 16 larger squares have been counted.
9. Clean the counting chamber, place in it a fresh drop of the diluted blood, and repeat the above counts.
10. Calculate as for the 'red counter.'

## 6.—MAKING BLOOD FILMS.

Apparatus, &c. — Perfectly clean cover slips, surgical needle, fine forceps, clamp forceps.

1. Be sure the cover slips are absolutely clean. (1.)
2. Obtain a drop of blood. (2.)
3. Pick up a cover slip with the forceps, and fix a second slip by one edge in a pair of clamp forceps.
4. Touch the summit of a drop of blood with the first cover slip.
5. Place the cover slip, immediately, face downward, upon the second cover slip: the blood will at once spread as a thin film. (*Do not press the cover slips, but let the film of blood spread of its own accord.*)
6. Immediately take the cover slips up, and, holding the edge of the lower one between the clamp forceps, take the edge of the upper one between a second pair of forceps and *slide* them apart, *being careful not to pull or press upon the film in any way.*

Note.—*If the films are not required for use at once, they may, when dry, be kept between layers of blotting paper.*

## 7.—FIXING BLOOD FILMS BY 'WET METHOD.'

1. Prepare blood films as usual. (6.)
2. Hold for 15 seconds over strong formalin vapour.
3. Place at once, *before the film dries*, into absolute alcohol for 20 mins.
4. Stain by Jenner's method, without allowing the film to dry during any part of the process.

Note.—*This method is especially suitable for staining films of bone marrow. The method is fully described by the Hon. G. Scott, in the 'Journal of Pathology and Bacteriology,' Vol. VII., page* 131.

## 8.—STAINING BLOOD FILMS BY JENNER'S METHOD.

Apparatus, &c.—Jenner's stain (xxxiii.), forceps, watch-glasses, beaker, slides, and labels, chemically pure distilled water.

1. Prepare a film in the usual manner. (6.)
2. To fix the film, dry it in the air.
3. Place the cover slip surface upwards upon a clean piece of blotting-paper, pour on it two or three drops of Jenner's stain, and cover it at once with a watch-glass to prevent evaporation.
4. Stain for from 2 to 4 minutes.
5. Wash well in pure distilled water until a delicate pink blush replaces the dirty blue colour of the film.
6. Blot quite dry.
7. Mount in Canada balsam.

Note.—*The bottle containing Jenner's stain must always be kept tightly stoppered, otherwise the stain will be spoilt. It is of the utmost importance that the distilled water should be quite pure and fresh, and that the beaker containing the water should be quite clean. If pure distilled water cannot be obtained, use filtered rain water.*

*If these directions are carefully observed, the films stained by this method will always be perfect, and most beautiful specimens obtained without the slightest trouble.*

## 9.—STAINING BLOOD FILMS WITH EOSINE AND METHYLENE BLUE

Apparatus, &c.—Alcoholic eosine (ii.), Löffler's methylene blue (xxxv.), strong formalin in wide-mouthed stoppered bottles, Bunsen burner, fine forceps, beaker, large capsule.

1. Prepare blood films as usual. (6.)
2. Fix the films by holding them *surface downwards* in strong formalin vapour for $\frac{1}{2}$ minute.

3. Allow the films to dry in the air under a large capsule.
4. With the forceps hold the film in the solution of eosine for 20 to 60 seconds.
5. Wash in water, blot, and pass three times through the flame.
6. Stain in Löffler's methylene blue for 15 seconds.
7. Wash in water, blot, and *mount in water*.
8. Examine under a high power.
9. If properly stained, float carefully off the slide, blot, dry, and mount in Canada balsam.

NOTE.—(*a*) *If the film is under-stained with methylene blue, put it back again into the stain.*

(*b*) *If it is over-stained with methylene blue, dip it in* 1% *acetic acid* (*in and out*) *and wash in water.*

(*c*) *If it is under- or over-stained with eosine, begin with a new film.*

*Jenner's stain is much better than the above method, and should always be used in preference if it can be obtained.*

## 10.—STAINING BLOOD FILMS BY EHRLICH'S METHOD.

APPARATUS, &c.—Drying oven, temperature 110°, or copper foil; Ehrlich triacid stain (xviii.).

1. Prepare blood films as usual. (6.)
2. Dry in the air.
3. Fix them in a drying-oven or on a piece of copper foil at a temperature of 110° for 30 minutes.
4. Place the cover slip surface upwards upon a piece of clean blotting-paper, and with a glass rod allow 2 or 3 drops of Ehrlich triacid stain to run over the cover slip.
5. Stain from 3 to 5 minutes.

6. Wash in water and blot dry.
7. Mount in Canada balsam.

Note.—*The success of this method depends greatly on the stain used, and great care must be taken to obtain it from a trustworthy source.*

*A good substitute for a drying oven is a piece of stout copper foil, about* 12 *in. long and* 2 *in. broad; one end is heated on a tripod over a Bunsen flame, and in a few minutes the temperature towards the other end will remain constant. The exact temperature for fixing the films will be found by dropping a little xylol on the copper foil, and taking that point at which this liquid just boils.*

## 11.—MICROSCOPIC EXAMINATION OF THE STAINED FILMS.

First examine the stained film so as to obtain a general impression of the relative number of leucocytes present.

Note (1) the presence of nucleated red corpuscles and their varieties, and the proportion of 'blood plates.'
(2) the shapes of red blood corpuscles.
(3) the varieties of leucocytes present.
(4) the preponderance of eosinophile cells, &c.
(5) the presence of parasites.

### Relative Count of the Leucocytes.

1. Having obtained a good general impression, fix the specimen on a mechanical stage.
2. If there is leucocytosis use an oil immersion; if not, an ordinary dry high power will answer.
3. Count field after field, and note the number of different leucocytes in each.

4. Count at least 500 leucocytes.
5. Finally calculate the percentages of the different varieties.

NOTE.—*While counting the white blood cells, always be on the look-out for changes in the red blood corpuscles (any changes in shape or size, or the presence of nucleated red blood corpuscles).*

*A mechanical stage is very convenient, but not absolutely necessary.*

## 12.—CLASSIFICATION OF LEUCOCYTES.

### LEUCOCYTES PRESENT IN NORMAL BLOOD.

| *Kanthack.* | | *Ehrlich.* | | |
|---|---|---|---|---|
| *Finely granular eosinophile* | = | *Polynuclear (neutrophile)* | = | (70-72°/o) |
| *Coarsely granular eosinophile* | = | *Eosinophile* | = | (2–4°/o) |
| *Lymphocytes* | = | *Lymphocytes* | = | (22–25°/o) |
| *Large uninuclear (hyaline)* | = | *Large mononuclear* | = | (2–4°/o) |
| *Atypical large uninuclear* | = | *Transitional forms* | | |
| *Basophile cells* | = | *Mast cells* | = | ( 0·5°/o) |

### CELLS WHICH MAY BE PRESENT IN MORBID BLOOD.

*Myelocytes.*

*Eosinophile myelocytes.*

*Red blood corpuscles showing :—*

*Polychromatophile degeneration.*

*Poikilocytosis.*

*Nucleated red blood corpuscles :—*

*Microblasts.*

*Normoblasts.*

*Megaloblasts.*

*The figures in brackets denote the normal limits.*

*Finely granular eosinophile cells or polynuclear leucocytes* are cells with irregular deeply staining nuclei. The protoplasm when stained with eosine presents a finely granular appearance, and with Ehrlich's triacid stain gives a neutrophile granulation. These cells, in films which have not been carefully prepared, appear to vary much in size and shape. The form which in these cases gives most trouble to the student is the 'exploded' cell. This appears as a large, more or less irregular nucleus, often staining faintly and somewhat resembling a large atypical lymphocyte; the fine granules may be so scattered that they appear absent, but careful search will usually show some of them lying as discrete granules between the surrounding corpuscles. It is, however, more common to find the nucleus pressed out of shape and surrounded by finely granular particles occupying two or three times the space of a normal cell. These cells are, with a little experience, readily distinguished from coarsely granular eosinophiles and also from myelocytes, but in cases of doubt the cells are nearly always finely granular eosinophile.

*Coarsely granular eosinophile cells.*— Cells with irregular nuclei, which as a rule do not stain so deeply as the nuclei of the finely granular eosinophile cells. The protoplasm shows large, coarse, round granules, which take a bright and deep pink with eosine, and stand out clearly from the surrounding cells. In carelessly made films 'exploded' forms may be found. These cells are easily recognised, and in any case where the student is in doubt the cell is almost certainly a finely granular eosinophile.

*Lymphocytes* 'are small cells, as a rule approximating in size to red blood corpuscles. Their body is occupied by a large round homogeneously stained nucleus, centrally situated.' Within the nucleus one or more nucleoli are often seen. The protoplasm surrounding the nucleus often stains more deeply than the nucleus.

*Large mononuclear leucocytes* are cells two or three times the size of a red blood corpuscle; they show a large oval nucleus, as a rule situated towards one side of the

cell. The nucleus stains feebly, and the protoplasm of the cell is fairly abundant.

*The transitional forms* of these cells resemble the preceding, but the nucleus often shows a deep notching, and resembles an hour-glass or kidney shape. The nucleus also stains a little more deeply than that of the atypical large mononuclear.

*Basophile cells* are cells which show with Jenner's stain a faintly stained nucleus surrounded by deeply stained basophile granules. In triacid preparations the granulation is unstained, and they appear as polynuclear cells free from granules.

*Myelocytes*, as a rule, are large cells, with a large rounded nucleus faintly stained, and surrounded by a finely granular protoplasm, which with Jenner's stain often shows a bluish-pink tinge. These cells vary in size and in granulation, but after a little experience there is no difficulty in recognising them.

*Eosinophile myelocytes* are larger, as a rule, than the coarsely granular eosinophile, but medium and small types are also found. The nucleus is often rounded, and larger than that of the coarsely granular eosinophile.

*Red blood corpuscles*, in well-prepared specimens, keep their natural size and shape. With all stains the hæmoglobin alone attracts the dye, and a practised observer can (if the film has been prepared by himself) form an opinion as to the relative amount of hæmoglobin present in each cell from the colour of the stained corpuscles.

*Polychromatophile degeneration* is a term applied to red blood corpuscles which stain a mixed colour. In typical specimens cells are often seen in all stages, from a pure red normal corpuscle, through a faint bluish-red, to fairly intense blue with scarcely a trace of red. These changes are easily seen and readily recognised.

*Poikilocytosis* is a name given to conditions where many of the red blood cells show marked alteration in size and shape. They show most irregular forms, and the change is at once noticed in any specimen, but care must be taken not to mistake the condition for that produced by unskilful preparation of the film, when the cells may be pulled or pressed out of shape. The examination of an unstained fresh specimen of blood will always clear up any doubts.

### NUCLEATED RED CORPUSCLES.

*Normoblasts* are red corpuscles of the size of a normal non-nucleated cell. The protoplasm, as a rule, stains as a normal corpuscle, but possesses a distinct deeply stained nucleus (occasionally more than one). The nucleus is always easily recognised, but students are warned against mistaking particles of stain or stained foreign matter, which is often present in films; in cases of doubt, several corpuscles should be found which show the nucleus, and only those of which there can be no doubt should be recorded.

*Megaloblasts* are cells from two to four times as large as a normal red corpuscle. Their protoplasm constitutes by far the chief portion of the body of the cell, and often shows polychromatophile degeneration to a greater or less degree. The nucleus is larger than that of the normoblasts, but does not occupy a relatively large portion of the cell. It stains more faintly than the nucleus of the normoblast, and may be so small that it is missed by an unpractised observer.

*The microblast* is very seldom found, and is recognised as being distinctly smaller than the normoblast.

NOTE.—*The student should note that a large variety of forms of leucocytes will be met with, especially in films which have been made by unskilled workers. These give rise to much doubt and difficulty, especially the atypical forms of lymphocytes and large mononuclear cells. Drawings should be made of all cells of which there is doubt, and these kept for future reference. If this is done the difficulties will diminish with each examination.*

Students will find *The Histology of the Blood*, by Ehrlich and Laazrus, trans. by Myers, a most useful book for reference. It contains more information on the subject than any other book of its size published.

## Scheme for Making an Examination of Blood.

1. Examine the blood unstained. (3.)
2. Estimate the hæmoglobin. (4.)
3. Estimate the specific gravity. (13.)
4. Estimate the number of red blood corpuscles and leucocytes. (5.)
5. Calculate the ratio of $\frac{\text{Hb}\%}{\text{R.B.C.}\%}$

   Thus, if the R.B.C. = 4,000,000, then assuming 5,000,000 to be normal, the R.B.C.% = 80; if the Hb% was 50, the ratio $\frac{\text{Hb}\%}{\text{R.B.C.}\%} = \frac{50}{80} = \frac{5}{8} = 0{\cdot}625$.

   This is also called the *colour index.*
6. Stain films and make a relative count of leucocytes present, noting any changes in the red cells, the presence of parasites, &c.

## Scheme for Recording a Blood Examination.

*Hb.* = %
*Sp. gr.* = = % *Hb.*
*R.B.C.* = = 1 *W.B.C.* to *R.B.C.*
*W.B.C.* =
$\frac{Hb\%}{R.B.C.\%} =$ ∴ *Colour index* =

*Relative count* (*in per cent.*).
*Finely granular eosinophile* = %
*Coarsely granular eosinophile* = „
*Lymphocytes* = „
*Large mononuclear* = „
*Transitional forms* = „
*Basophile* = „
*Myelocytes* = „
*Eosinophile myelocytes* = „
*Nucleated red corpuscles* = „

*Variations in shape of red corpuscles.*
*Presence and proportion of 'blood plates.'*
*Parasites present.*
*Remarks.*

## 13.—ESTIMATION OF SPECIFIC GRAVITY OF BLOOD (HAMMERSCHLAG).*

APPARATUS, &c.—Urinometer-glass, urinometer, chloroform, benzol, needle, capillary pipette, glass rod.

1. Take chloroform and benzol, mix these liquids in a urinometer-glass in such proportions that the specific gravity of the mixture is about 1059, *i.e.*, that of normal blood.

2. Puncture the ear, draw a drop of blood into a capillary pipette, and blow it out into the mixture. The blood will not mix with this liquid, but floats as a red bead.

3. If it sinks to the bottom, add chloroform; if it rises to the top, add benzol. When the right density is reached, the bead of blood will remain suspended in the liquid.

4. When this point is reached, take the specific gravity of the liquid, and you will have the specific gravity of the blood that just floats in it.

5. Add a fresh drop of blood to make sure that the specific gravity is correct.

NOTE.—*The following precautions are needed:—The urinometer-glass must be perfectly dry and clean; add the chloroform or benzole a few drops at a time, and after each addition stir thoroughly with a glass rod; avoid having any air adherent to the blood-drop; the whole process must be carried out as quickly as possible.*

*The mixture may be used over and over again by simply filtering it after use.*

*This method is most convenient, and with a little practice rapid and sure.*

---

* This method is useful to check the results obtained by the Von Fleischl hæmometer, and also when this instrument is not obtainable, as it is thereby possible, in cases where there is no dropsy or œdema, to calculate the amount of hæmoglobin present.

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*It is possible to calculate the relative amount of hæmoglobin present by means of the following table :—*

| Specific gravity. | Hæmoglobin. |
|---|---|
| 1·033–35 | 25–30 per cent. |
| 1·035–38 | 30–35 ,, |
| 1·035–40 | 40–45 ,, |
| 1·045--48 | 45–55 ,, |
| 1·048–50 | 55–65 ,, |
| 1·050–53 | 65–70 ,, |
| 1·053–55 | 70–75 ,, |
| 1·055–57 | 75–85 ,, |
| 1·057–60 | 85–100 ,, |

## 14.—BACTERIOLOGICAL EXAMINATION OF BLOOD

Apparatus, &c.—Syringe, sterilised citrate solution (xv.), small sterilised Petri's capsule, two agar-agar tubes, broth tube.

*It is often necessary to make a bacteriological examination of the blood from patients supposed to be suffering from septicæmia or pyæmia, etc. To do this properly, the following method is probably the best :—*

1. Prepare the arm of the patient, as for operation, over the median basilic vein at the bend of the elbow two or three hours before the examination is to be made.
2. Prepare an absolutely sterile syringe with sharp needle. The syringe must be carried in a large sterilised test-tube, plugged with cotton wool.
3. Have also ready :—
    - (*a*) Sterile solution of sodium citrate in a plugged test-tube.
    - (*b*) A small sterilised Petri's capsule.
    - (*c*) Two agar-agar tubes.
    - (*d*) A broth tube.

4. On reaching the bedside, place the patient's arm in a good light, and, having your hands absolutely clean, remove bandages with the exception of the last piece of gauze.
5. Place all the apparatus ready for use.
6. Tie a bandage round the patient's arm just below the axilla, so as to obstruct the venous circulation but not the arterial.
7. Take the syringe from its tube and fit the parts together if this has not already been done.
8. Pour some of the sterilised citrate solution into the sterilised Petri's capsule. *Avoid any unnecessary exposure to the air.*
9. Half fill the syringe with citrate solution from the capsule.
10. Hold the syringe firmly, remove the last piece of dressing, and insert the needle into the largest vein at the bend of the elbow.
11. Gently withdraw the piston of the syringe and the blood will flow readily into the cylinder, mixing with the citrate solution.
12. Withdraw the needle, and, while withdrawing it, apply pressure over the puncture with a piece of sterile gauze.
13. Let your assistant keep his finger over the puncture, whilst you as rapidly as possible mix the blood and the citrate solution together, by rotating the syringe; this will prevent clotting.
14. Bandage the patient's arm carefully, though as a rule no hæmorrhage takes place.
15. With the diluted blood in the syringe
    - (*a*) Inoculate two agar-agar tubes. (24, A.)
    - (*b*) Inoculate a broth tube. (24, B.)

16. Incubate these two tubes 24 to 48 hours, and examine for micro-organisms.

Note.—*The bacteriological examination of the blood often gives negative results, because small quantities are taken. By the above method* 5–10 *c.c. may be obtained with ease if the syringe is large enough, and it is attended by no more pain than that caused by an ordinary hypodermic injection: it is perfectly safe if ordinary precautions are used, and with a little practice quite easy. The only difficulty is to get the needle into the vein, but if the arm is well constricted above the elbow, and a sharp needle is used, it will usually enter the vein at once, and the blood flow readily into the syringe. If the blood serum only is required the citrate solution need not be used. In some cases of severe anæmia the circulation is so feeble that the blood does not distend the veins after constriction. This difficulty is overcome by lowering the arm, and raising the foot of the bed.*

## 15.—SERUM DIAGNOSIS IN ENTERIC FEVER (WIDAL-GRÜNBAUM TEST).

Apparatus, &c.—Six small test tubes with feet, 18-hour-old typhoid culture, platinum loop, broth tubes, capillary pipette, paraffin wax.

### A.—Method of Collecting Blood.

1. Prick the lobe of the ear, or the tip of the finger.
2. By gentle pressure obtain a large drop of blood, and collect it in a small capillary pipette.
3. Seal the ends of the pipette in the flame; the pipette may now be carried any distance.

### B.—Dilution of Blood.

4. Make a mark with ink on the pipette at a point corresponding to the level of the blood, and break off the ends of the pipette.

5. Blow the blood out into a small sterile test-tube, and with the same pipette add four times the amount of sterile broth, thus obtaining a dilution of 1 in 5.
6. Gently shake the tube.

### C.—PREPARATION OF SUSPENSION OF TYPHOID BACILLI.

7. Place a little sterile broth into a small test-tube.
8. Add to this 2 or 3 loops of a fresh agar-agar culture of typhoid bacilli, not more than 24 hours old, and obtain a turbid suspension.
9. Stir with the loop, so as to obtain a uniform suspension of the bacilli; *sterilise the platinum loop.*
10. Examine a drop microscopically, in order to make certain that the suspension contains no clumps, and that the bacilli are actively motile.

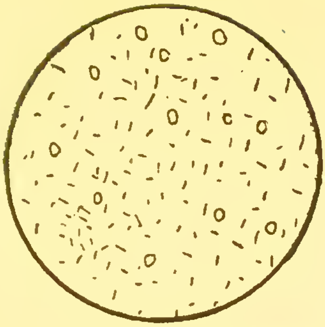
### D.—PREPARATION OF SLIDE.

11. With the pipette place a drop of the diluted blood on a slide.
12. With a new pipette add a drop of the suspension of typhoid bacilli to the drop of blood, so that the blood is now diluted to 1 in 10.
13. Mix the drops with a sterile platinum needle, and place a cover glass on the slide.
14. Paint a ring of melted paraffin around the cover glass.

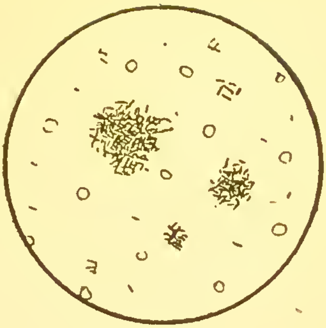
### E.—EXAMINATION.

15. Place the specimen under the microscope (high power) and examine it from time to time. The red corpuscles assist in obtaining the right focus.
16. Watch for loss of motility on the part of the bacilli, and for clumping or agglutination.
17. In a positive case this should occur within an hour.

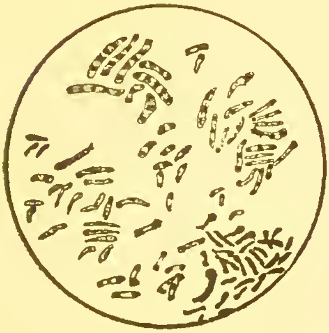
# PLATE II.



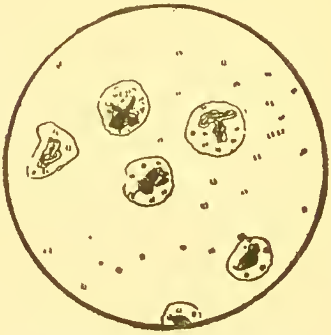
A. Serum test, non-clumping.



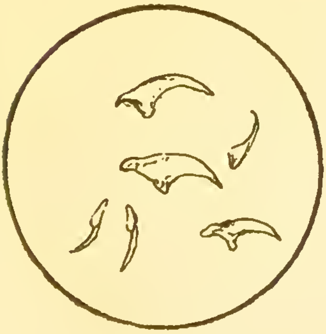
B. Serum test, clumping.



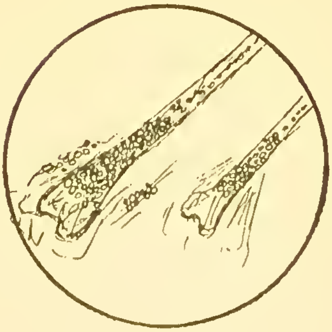
C. Types of diphtheria bacilli.



D. Diplococci in pus.



E. Hydatid hooklets.



F. Ringworm.

18. If the reaction is complete, take the small test-tube containing the diluted blood, and with the first pipette dilute the blood still further by adding 10 pipettes of broth, being careful to draw the broth up to, and not beyond, the mark made to show amount of blood taken; this will give a dilution of 1 in 15.

19. Proceed as before. The blood, when mixed with the suspension of typhoid bacilli, is now diluted 1 in 30.

20. Then dilute the blood still further by adding, as before, 10 times the proper amount of broth, and put up another drop. This specimen is diluted 1 in 50.

21. Examine these two drops under the microscope as before.

NOTE.—(*a*) *It is important to pay attention* (1) *to the degree of dilution;* (2) *to the rapidity of the reaction.*

*The dilution should never be less than* 1 *in* 20, *and the reaction should appear in one hour.*

(*b*) *If the reaction is negative* (Pl. II., A), *the test must be repeated daily or every other day until the reaction appears or patient recovers; it may be delayed for some weeks.*

(*c*) *The smallest drop of blood suffices for the test, the blood may be collected on blotting paper if no pipettes are at hand. This must of course be soaked in water before use.*

(*d*) *Should the blood clot in the pipette so that it cannot be blown out, break off the end of the pipette with the clotted blood in it, and drop it in the small test-tube. Then add approximately four times the amount of broth and shake, in order to dilute the blood thoroughly, and proceed as before.*

(*e*) *Clumping, or agglutination, is a term applied to the typhoid bacilli when they adhere to each other and form clumps of bacilli.* (Pl. II., B.)

### A.—ORDINARY METHOD.

APPARATUS, &c.—Tripod and sand-bath, Bunsen burner, watch-glasses, carbol fuchsine (xi.), wide-mouthed bottles containing water, 75 per cent. spirit, 25 per cent. hydrochloric acid, Löffler's methylene blue (xxxv.), slides and cover slips, Canada balsam, blotting-paper.

1. Pour the sputum into a small glass dish, which is placed upon a black surface.
2. With a pair of fine forceps pick out such fragments as appear purulent, caseous, or necrotic.
3. Rub one of these fragments over a clean cover glass.
4. Place a second cover glass upon this, and, holding them between two folds of blotting-paper, press them together.
5. Gently slide them apart and allow the films to dry in the air, protecting them from dust by placing them, *surface downwards*, in a watch-glass. *Burn the blotting-paper.*
6. When dry, pass the films through the flame *surface upwards.*
7. Float the films, *surface downwards*, on a warm carbo fuchsine solution for 2 to 5 minutes.
8. Take one of them out, leaving the other in the stain for subsequent examination.
9. Wash rapidly in water.
10. Decolorise in 25% hydrochloric acid, by dipping the films in the acid just long enough to discharge the red colour.
11. Wash immediately in 60 to 70% spirit, till no more red comes away.
12. Wash in water.
13. Counter-stain in Löffler's methylene blue (20 to 50 seconds).
14. Wash in water, blot, and dry in air.

15. Mount in Canada balsam.
16. Examine with oil immersion.

*If tubercle bacilli are found, no other method is required. If no tubercle bacilli are found proceed as in* B *or* C.

### B.—VAN KETEL'S METHOD.

APPARATUS, &c.—In addition to that required for A: Small flask, small beaker, liquid carbolic acid, separating tubes, centrifugalising machine, pipette, chloroform, 10% solution of caustic soda, egg albumen, burette or conical flask if no centrifugalising machine is available.

1. Pour 100 c.c. of sputum into flask.
2. Slowly add 5 c.c. of liquid carbolic acid, and shake thoroughly.
3. Pour the contents of the flask into separating tubes and centrifugalise or stand aside for 24 hours in a burette and then examine sediment.
4. Remove with a pipette a little of the sediment from the lower layers, and prepare films as in A.
5. Dry the films in the air and pass through the flame.
6. Wash them in chloroform for 1 to 3 minutes.
7. Quickly wash in water, blot, and again pass through the flame.
8. Then stain with carbol fuchsine and counter-stain with methylene blue as above.

### C.—BIEDERT'S METHOD.

*This method is specially useful when the sputum contains much mucus.*

1. Take 15 c.c. of the sputum and mix with 30 c.c. of water in a small beaker.
2. Constantly stirring, add a little 10% caustic soda (4 to 8 drops).
3. Then slowly boil, while adding gradually 60 to 90 c.c. of water until the mixture acquires a thin consistence.
4. Now either let the liquid stand in a conical glass for 2 or 3 days, or better still, centrifugalise.

5. The sediment is mixed with a little egg albumen and then examined and stained for tubercle bacilli like ordinary sputum with this difference, that the films must be left in the warm carbol fuchsine solution fully 5 to 8 minutes.

*If, as often happens, only a little sputum is available, proceed as follows :—*

(i.) Shake up 1–3 c.c. of it with 6–10 volumes of 0·2% caustic soda in a test tube for 3 mins.

(ii.) Boil this mixture up several times in a small beaker.

(iii.) Centrifugalise and proceed as above.

*This method is also useful for the examination of pus for tubercle bacilli and whenever there is but a small supply of material.*

NOTE.—*The examination of sputum for tubercle is an important means of diagnosis, and it is essential that every student should become quite familiar with the methods. If bacilli are plentiful the rest is easy ; but if they are few in number the examination becomes one of the most irksome duties a student or medical man can undertake. Nothing but careful collection of the sediment, after breaking down the viscid mucus, and a careful examination of a large number of films, day by day, will make it possible for a reliable negative report to be given. Every portion of the stained film must be gone over, field by field, with a one-twelfth immersion, and in the event of no bacilli being found, the report can only be to that effect, viz., 'No tubercle bacilli found.' Experience is, however, a great help, and well-trained observers seldom fail to find bacilli if present. The animal test is by far the most satisfactory when no bacilli are found, and should always be made in cases where a definite report is essential, but in England it can only be undertaken by a holder of a licence for vivisection. The test consists in injecting a small quantity of the suspected material into the subcutaneous tissues of the thigh of a guinea pig, killing the animal in from 4 to 6 weeks, and examining the organs for a deposit of tubercle.*

## 18.—EXAMINATION OF SPUTUM FOR MICRO-ORGANISMS AND LEUCOCYTES.

### A.—Staining by Löffler's Methylene Blue.

Apparatus as in **(25)**.

1. Prepare a film as in **17.**
2. Place the film in Löffler's methylene blue 5 minutes.
3. Wash in water.
4. Blot quite dry.
5. Mount in Canada balsam, and examine under a high power.

### B.—Staining by Gram's Method.

Apparatus as in **(25)**.

1. Prepare a film as in **17.**
2. Stain as in **25.**, C.

### C.—Staining for Eosinophile Cells.

Apparatus as in **(8)**.

1. Prepare a film as in **17.**
2. Stain as in **8.**

Note.—*The sputum must be quite fresh if the eosinophile granules are to be demonstrated.*

# DIPHTHERIA.

## 19.—CLINICAL DIAGNOSIS OF DIPHTHERIA.

### A.—To Make a Swab.

1. Take a piece of straight iron wire about 8 in. long, and the thickness of a knitting needle, wrap tightly around one end of this several thin layers of cotton wool so that about one inch of the lower end, including the point, becomes covered.
2. Place the end covered with the wool into a long, thick-walled test tube, leaving the other end outside, plug the mouth of the tube with cotton-wool plug, and sterilise in a hot-air steriliser.

Note.—*Several of these should be kept ready for use. If a sterile swab is not at hand, a stout platinum loop will answer.*

### B.—Taking a Culture from a Case of suspected Diphtheria.

1. Place the patient in a good light, and examine the tonsils and fauces for clinical signs, using, if necessary, a tongue depressor.
2. Take a sterile swab and rub it thoroughly over the tonsils and fauces, especially over any suspicious patch of membrane, and return it to its tube.
3. Rub the infected swab on the surface of Löffler's serum, or other serum medium, and incubate in a warm incubator. Examine microscopically as soon as any colonies are seen (often within 12 hours).

### C.—To Examine Cultures for Diphtheria Bacilli.

1. Examine the culture within 20 hours, and if there is a growth proceed as follows; if no growth is found, replace the culture in the incubator, and examine the next day.
   (*a*) Examine the colonies with the naked eye, or preferably with a hand lens.
2. With a straight sterile platinum needle, flattened at the point, pick up a portion of one of the suspicious colonies, and prepare
   (*b*) An unstained specimen (25, A).
   (*c*) A specimen stained with methylene blue (25, B).

3. Examine them for bacilli; if no bacilli are present, or bacilli which are evidently not diphtheria, make preparation from other colonies until you find bacilli resembling diphtheria, or until you are satisfied that no colonies of diphtheria bacilli are on the media; in the *latter* case reincubate the tube till the next day and then examine again.
4. If a suspicious colony is found proceed as follows:—
    - (*d*) Make another preparation, and stain by Neisser's method (25, E), and note if any beaded forms are seen.
    - (*e*) Proceed to isolate a pure culture as described in (24, D).
    - (*f*) Make a microscopical preparation (25, B) to see if suspension is pure.
    - (*g*) Inoculate from the suspension—
        - (1) A glucose broth tube, to test the capacity for forming acid (24, E).
        - (2) A Löffler's serum tube, to be sure a pure culture is obtained.
5. Incubate these in a warm incubator, and examine.
    - (*h*) Within 20 hours examine the serum tube by Neisser's method, and note if any beaded forms are seen. (25, E.)
    - (*i*) At the end of 48 hours test the reaction of the glucose broth tube, and note if acid or alkaline (24, E), previously making a microscopic examination to see if culture is pure.

*For Report, if—*

(1) *The case is clinically diphtheria;*
(2) *The culture shows bacilli resembling diphtheria bacilli in shape and arrangement;*
(3) *The bacilli show beading by Neisser's method;*
(4) *The glucose broth gives an acid reaction at the end of 48 hours;*

*then a Diagnosis of Diphtheria may be made.*

The bacteriological examination of a case of suspected diphtheria requires considerable experience and care if a definite opinion is to be given, and in many cases it is impossible to give a positive diagnosis without isolating a pure culture of the suspected bacillus, and certainly not without previous experience with cultures which have been tested on animals.

The student, however, and medical men who see the clinical aspects of the case, can make a very *safe* report if the work is carefully carried out. The above scheme shows how this should be done.

As a rule, the culture is made overnight, and examined the next morning, and if bacilli resembling diphtheria bacilli are found, the case should be reported as '*suspicious;*' in many cases it can be reported as '*certainly diphtheria*,' especially if it gives beading with Neisser's stain. To make the diagnosis more certain, the colony should always be isolated and tested with glucose broth, and if at the end of 48 hours this is acid, the diagnosis is confirmed; if alkaline, a mistaken diagnosis has probably been made.

With Neisser's stain it is important that the culture should not be more than 20 hours old, as, if older, other organisms may be mistaken for diphtheria bacilli—*e.g.*, Hoffman's bacillus.

## 20.—CLASSIFICATION OF TYPES OF DIPHTHERIA BACILLI.

*Diphtheria bacilli characteristic types.*—Diphtheria bacilli generally show on cultivation a 'characteristic arrangement' and a variety of form (Pl. II., C). This varies with the individual organism, with the kind of media used, and also with the age of the culture. Skilled observers recognise many types, but for the purpose of clinical diagnosis the types seen in recent cultures can be classified as follows:—

1. *Oval bacilli with an unstained septum.*—These appear to be young forms, and other forms are always present in the same culture.
2. *Long faintly stained irregularly beaded bacilli.*—This is a curved bacillus of irregular width, staining faintly, with one or more darkly staining rounded dots, often terminal, sometimes central.
3. *Segmented bacilli.*—These bacilli vary in length; they are curved and of irregular thickness; they stain darkly with several narrow unstained or faintly stained linear intervals, and one of the roughly rectangular segments is usually larger than the others.
4. *Regularly beaded bacilli* (*streptococcal forms*).—These appear as a row of dots at regular intervals in a pale bacillus; if faintly stained the dots alone may be seen, and the bacilli may be taken for streptococci; but the regular and somewhat stiff curve they assume, the uniformity of their length, and finally their 'arrangement,' will suggest their true nature.
5. *Uniformly stained bacilli.*—These stain so uniformly that it is difficult to see any trace of segmentation or granulation.

This classification is taken from an interesting description by Dr. Louis Cobbett in the *Journal of Hygiene*, Vol. I., No. 2, 1901.

# RINGWORM.

## 21.—EXAMINATION OF HAIRS FOR RINGWORM.

APPARATUS, &c.—Watch-glasses, slide and cover slips, 10% causti potash, ether, paraffin wax, carbol-gentian-violet (xii.), aniline oil and xylol (v.), Farrant's medium (xx.), Canada balsam, blotting-paper.

### A.—MACERATION WITH CAUSTIC POTASH.

1. Remove a number of hairs, and soak them in ether for a few minutes in a watch-glass.
2. Then fix them on a clean glass slide with a little melted paraffin, which, of course, must not be applied to the roots.
3. Now pour a few drops of 10% caustic potash upon the hairs, and allow them to macerate for some minutes.
4. Remove the caustic potash carefully with blotting-paper, and wash the hairs with water.
5. Remove the water with blotting-paper, and then mount in Farrant's solution.
6. Examine with ordinary high power for mycelial threads and spores.

### B.—STAINING BY MEANS OF WEIGERT'S METHOD.

1. Remove a number of hairs and soak them in ether for a few minutes.
2. Then fix them on a clean slide with melted paraffin, leaving the roots free.
3. Filter a few drops of carbol-gentian-violet upon the hairs.
4. After 5 to 10 minutes remove the stain with blotting-paper, and drop a little Weigert's iodine upon the hairs.

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5. After half a minute remove the iodine with blotting-paper, and decolorise with aniline oil and xylol in the ordinary manner, watching the hair all the time under a low power.
6. When properly decolorised wash thoroughly with xylol.
7. Remove the xylol with blotting-paper; remove the paraffin, and mount in Canada balsam.

Note (*a*) *the size of the spores: microspores or megalospores;* (*b*) *the arrangement of the mycelium: endothrix or ectothrix.* (Pl. II., F.)

*Scrapings from the skin may be examined by the above method.*

# GONORRHŒA.

## 22.—CLINICAL DIAGNOSIS OF GONORRHŒA.

Apparatus, &c.—Slides and cover glasses, carbol-gentian-violet (xii.), Gram's iodine (xxix.), alcohol, xylol, Canada balsam, Bismarck brown (viii.).

### A.—Staining Film with Carbol-Gentian-Violet.

1. Make films of the pus, as usual. (17.)
2. Stain with carbol-gentian-violet for 5 minutes.
3. Mount in water and examine, and if diplococci are present (Pl. II., D) stain the same film by Gram's method, counter-staining with Bismarck brown.

### B.—Staining Films by Gram's Method, and Counter-staining with Bismarck Brown.

1. Place the film in absolute alcohol for 2 minutes.
2. Stain it in carbol-gentian-violet for 2 minutes.
3. Remove superfluous stain with blotting-paper.
4. Pour on a little of Gram's iodine solution, leaving it for about half a minute (the specimen turns black).

5. Blot off the iodine solution.
6. Wash in alcohol till decolorised.
7. Wash well in water.
8. Stain *the same film* with dilute solution of Bismarck brown for 2 minutes.
9. Blot and dry.
10. Mount in Canada balsam.

NOTE.—*The gonococci are stained brown, other organisms blue.*

# PUS.

## 23.—MICROSCOPIC EXAMINATION OF PUS.

*Examine the pus by the following methods :—*

A. Place a small quantity on a glass slide under a cover slip, and examine unstained (25, A).

B. Make a similar preparation, and allow a little dilute acetic acid to run under the cover slip, and note the changes in the leucocytes.

C. Prepare films in the usual manner (25, B), place them 3 to 5 minutes in 10% acetic acid, wash in water, dry with filter paper, and pass once again through the flame.

*Stain the films* (C) *by the following methods :—*

(*a*) Methylene blue. (25, B.)

(*b*) Gram's method. (25, C.)

(*c*) Eosin and methylene blue. (9.)

(*d*) Jenner's method. (8.)

NOTE.—*If the pus is to be examined for tubercle bacilli, stain as in* (17, C.).

# BACTERIOLOGICAL METHODS.

## 24.—INOCULATING CULTURE TUBES.

APPARATUS.—Media, platinum loop, Bunsen burner, litmus solution (xxxiv.), small test tubes.

### A.—MAKING SLANT CULTURES.

1. Take the material from which a culture is to be made and place it close at hand.
2. Take two slanted media tubes and singe the cotton-wool plugs.
3. Hold the tubes between the thumb and index finger of the left hand.
4. Sterilise the platinum loop, and, *when it is cool*, take up with the loop a minute quantity of the material to be examined.
5. Remove the plugs from the tubes, and hold them between the fingers of the *left* hand.
6. Introduce the loop into one of the culture tubes, and draw it three times along the surface of the medium; withdraw the loop.
7. Introduce the loop into the second tube *without re-charging it*, and draw it three times over the surface of the medium as before.
8. Replace the cotton-wool plugs into their respective tubes.
9. Sterilise the platinum loop in the flame.
10. Label the tube carefully, and place in the incubator.

### B.—MAKING A BROTH OR BOUILLON CULTURE.

Proceed as above, but introduce the platinum loop into the liquid medium, and agitate it slightly while in the medium to wash the material from the loop.

### C.—TRANSPLANTING COLONIES FROM ONE TUBE TO ANOTHER.

Proceed as in A, but hold the tube containing the colony to be transplanted between the thumb and index finger of the left hand by the side of the tube to which the colony is to be transplanted. Pick up the colony with the end of the platinum loop, and streak the tube as before.

### D.—TO ISOLATE COLONIES OF MICRO-ORGANISMS GROWING ON SOLID MEDIA.

1. Take a test tube containing sterile 0·7 % salt solution, and hold it in the left hand, with the tube containing the culture to be isolated.
2. Sterilise a straight platinum needle in the flame.
3. Remove the plugs from both tubes, and with the point of the platinum needle pick up a portion of the colony to be isolated.
4. Wash the end of the needle in the sterile salt solution, and so suspend the micro-organism in the liquid.
5. Make a cover-slip preparation without recharging the needle.
6. Replace the plug.
7. Sterilise the needle in the flame.
8. Stain the cover-slip preparation with methylene blue (25 B).

9. Examine carefully under a high power, and notice if the organism you wish to isolate is present, and also whether micro-organisms of other kinds are also there.
10. If, as far as can be ascertained by the aid of the microscope, the salt solution contains the organism you wish to separate and no other, then shake up the suspension, and inoculate the medium.
11. Incubate these sub-cultures, and always make a microscopic preparation from them after they have grown, to be sure that the sub-cultures are pure.
12. If the colony selected has proved not to be the right one, or if other micro-organisms have been taken up with those which it is desired to isolate, another salt solution tube and cover slip must be prepared, and the processes repeated again and again until success has been gained.
13. In some cases, where the original culture is overgrown, a salt solution tube must be sown with a mixture of the organism it is desired to isolate and the other forms of bacteria present, and of this suspension a single loop should be taken, and 3 or 4 slant tubes inoculated in succession. *Without recharging the loop* it is thus possible to obtain a number of discrete colonies, and from these to select the particular *micro-organism* you wish to isolate.

NOTE.—*The above method was designed by Dr. Louis Cobbett for the isolation of the diphtheria bacillus from impure cultures, and it is for this purpose that it has been described here. It is specially suited to the diphtheria bacillus, because it grows quickly, and the medium used favours it, but it is not suited for every form of micro-organisms, and it is not intended to replace plate cultures.*

*If sterile salt solution is not at hand, a broth tube will answer, but if many colonies are to be isolated the salt solution saves the medium.*

## E.—To Test the Reaction of a Glucose Broth Culture.

1. Ascertain that the glucose broth tube is alkaline before sowing it.
2. Make a culture of the organism in the glucose broth.
3. Incubate for 48 hours.
4. Examine microscopically by means of a stained specimen from the incubated tube, and be sure that the organism you wish to test is present, and no other; before staining the cover-slips, they should be passed through 2% acetic acid, and washed in water (25, B).
5. Take two small test tubes, and pour into each a little litmus solution.
6. Pour into one a small quantity of the glucose broth culture, and note if any change of colour occurs in the solution. This can be easily seen by comparing it with the solution in the other test tube.

Note.—*This test is especially useful in the diagnosis of diphtheria, as true diphtheria bacilli are acid producers in the presence of glucose, and therefore give an acid reaction.*

*If for any reason the culture of diphtheria bacilli grows badly, the acid formation may be delayed.*

## 25.—MICROSCOPICAL EXAMINATION OF CULTURES.

Apparatus.—Microscope, platinum loop, Bunsen burner, blotting-paper, stains, spirit, aniline oil and xylol (v.), Weigert's (liii.) and Gram's iodine solutions (xxix.), carbol-gentian-violet (xii.), Neisser's stain (xl.).

### A.—Examination by means of Unstained Specimens.

1. Examine the culture carefully in a good light, and note the size, shape, colour, and number of the colonies.
2. Fix upon a colony you wish to examine, singe the plug of the tube.
3. Place a small drop of water upon a clean cover slip lying on a piece of blotting-paper.
4. Sterilise the platinum loop in the flame, and *allow it to cool.*
5. Hold the tube between the thumb and index finger of the left hand, remove the plug, and hold it between the fingers of the left hand.
6. With the sterilised loop pick up a minute portion of the colony to be examined.
7. Replace the plug in the tube.
8. Spread the drop of water on the cover slip over the surface with the platinum loop, and thus make a suspension of the bacteria in the water on the cover slip.
9. Sterilise the platinum loop in the flame.
10. Pick up the cover slip with a glass slide.
11. Blot off excess of water with blotting-paper, *burn the blotting-paper.*
12. Examine with a high power.

### B.—To Make a Film for Staining Bacteria.

1. Proceed exactly as above from 1 to 9.
2. Allow the film to dry in the air under a watch-glass.
3. Pass the cover slip three times through the flame, film upwards.
4. Filter on to the cover slip a few drops of the stain and allow it to remain for 5 minutes.
5. Wash in water, blot dry, mount in Canada balsam.
6. Examine under a high power.

Note.—*The stains in general use are methylene blue, gentian violet, fuchsine, and Löffler's methylene blue.*

*If the film is made from a broth culture it should, after passing it through the flame, be dipped into 2% solution of acetic acid, and then washed in water before staining.*

### C.—To Stain Bacteria by Gram's Method.

1. Prepare a film as above from 1 to 9.
2. After it has dried, pass through the flame, and place it in absolute alcohol for 1 minute.
3. Without drying, remove it into carbol-gentian-violet for 5 minutes.
4. Blot off excess of stain on blotting-paper.
5. Place in Gram's iodine solution for 1 minute.
6. Blot off excess of iodine solution, and wash in spirit till no more blue colour comes away.
7. Wash in water, blot dry, and mount in Canada balsam.

### D.—To Stain Bacteria by Weigert's Method.

1. Prepare a film as above.
2. Dry in air, and pass three times through the flame.
3. Filter on to the film a little carbol-gentian-violet stain for 5 minutes.

4. Drain off excess of stain on blotting-paper.
5. Pour on to the film a little of Weigert's iodine solution; leave for 1 minute.
6. Blot between several folds of blotting-paper.
7. Wash with a mixture of aniline oil and xylol till no more blue colour comes away.
8. Wash in xylol, and mount in Canada balsam.

### E.—To Stain Bacilli by Neisser's Method.

1. Make a cover-slip preparation (**25** B).
2. Place it in Neisser's stain for 5–20 sec.
3. Wash in water.
4. Counterstain with Bismarck brown or Neisser's contrast stain, ½ min.
5. Wash, blot dry, and mount in Canada balsam.

Note.—*Neisser's method is used in the diagnosis of diphtheria. The bacilli must be grown on Löffler's serum media, and must be less than* 20 *and more than* 7 *hours old. True diphtheria bacilli, as a rule, show very definite polar beading, and often beading in the body of the bacillus. There are, however, exceptions to this rule; some cultures of true diphtheria bacilli show no beading, other cultures which turn out to be non-virulent bacilli show well-marked beading; but, in spite of this, the method is helpful in doubtful cases when combined with other tests, and every case giving well-marked beading, and showing bacilli of characteristic shape in cultures less than* 20 *hours old on Löffler's serum, should be regarded as suspicious until the contrary is proved.*

# EXAMINATION OF URINE.

## 26.—THE COLOUR OF THE URINE.

Observe by reflected and transmitted light the colour of the specimen of urine.

Shake the liquid, and notice the colour of any froth that may form.

### A.—The Colour due to Normal Pigments.

1. Urine of usual colour = an amber yellow.
2. Urine of lighter colour = a light straw.
3. High-coloured urine = a reddish yellow.

Note.—*Urine containing normal pigments may vary from an almost colourless to an orange-red liquid.*

*The normal pigments may be increased* (*a*) relatively, *as in concentrated urine;* (*b*) absolutely, *from increased metabolism.*

*The pigments are diminished in dilute urines, and in anæmia.*

### B.—The Colour due to Abnormal Pigments.

4. Bile pigment = a yellowish green.
5. Blood in urine = a bright red.
6. Blood in urine = a brownish black = 'smoky' urine.

Note.—*Urines containing* bile *occur in jaundice and may vary from a yellow to brownish-green colour: it is best seen by shaking the liquid, and examining the froth that forms. Urines containing much urobilin give a greenish froth on shaking. Urines containing* blood *vary from a light bright red to a deep brownish-black colour: the colour is best seen by transmitted light. The colour of urine may be changed by other pigments, e.g., melanin; and by drugs, e.g., carbolic acid, rhubarb.*

## 27.—NAKED EYE EXAMINATION OF URINARY SEDIMENTS.

### A.—Healthy Urine.

1. Specimen of healthy urine freshly passed
= *quite clear.*

2. Specimen of healthy urine after standing
= *delicate cloud.*

3. Specimen of concentrated urine
= *deposit of urates.*

4. Specimen of urine with deposit of mucus
= *delicate, almost transparent, cloud.*

### B.—Abnormal Urine with Organized Sediment.

5. Specimen of urine containing blood
= *faint bright-red colour.* (36.)

6. Specimen of urine containing blood
= *bright-red colour.* (36.)

7. Specimen of urine containing blood
= *brownish = smoky urine.* (36.)

8. Specimen of urine containing pus
= *thick and viscid sediment.* (38.)

9. Specimen of urine containing pus
= *slimy and translucent sediment.* (38.)

10. Specimen of urine containing pus and blood
= *reddish-brown sediment.*

### C.—Abnormal Urine with Unorganized Sediment.

11. Specimen of urine containing excessive deposit of urates
= *bright red* (41 C).

12. Specimen of urine containing deposit of phosphates
= *white* (41 C).

13. Specimen of urine containing deposit of uric acid
= *brick-red dust* (41 C).

## 28.—THE REACTION OF URINE.

Test the urine with red and blue litmus paper.

1. Normal urine freshly passed = *acid reaction.*
2. ,, after standing = ,,
3. ,, ,, 48 hrs. = *alkaline reaction (volatile).*
4. Urine from case of cystitis = ,, ,,
5. Urine alkaline when passed = ,, *(fixed).*

NOTE.—*With a (so-called) fixed alkali, if red litmus paper is used, the blue colour persists after drying. With a volatile alkali the blue colour fades and the red returns on drying with a gentle heat.*

## 29.—SPECIFIC GRAVITY.

APPARATUS.—Urinometer, cylindrical glass vessel, filter paper, and funnel.

1. If the urine is turbid, filter.
2. Fill the glass cylinder three-quarters full with urine.
3. If any froth appears on the surface, remove it with filter paper.
4. See that the urinometer is clean and dry.
5. Sink the urinometer gradually into the liquid until it floats freely.
6. Read the scale where it cuts the lowest level of the concave surface of the liquid by bringing the eye to a level with, or below the surface of the urine.

NOTE.—*The specific gravity of healthy urine varies from* 1015 *to* 1020.

## 30.—DETERMINATION OF ALBUMEN.

Apparatus and Reagents.—Test-tubes and rack, Bunsen burner, filter paper, funnel, pipettes, concentrated nitric acid, acetic acid.

### A.—Heller's Ring Test.

1. Filter the urine if turbid.
2. With a pipette place 2 c.c. of nitric acid in a test-tube, taking care not to allow the acid to touch the sides of the tube.
3. With a fresh pipette take up 2 c.c. of the urine, and placing the end of the pipette close to the surface of the acid in the test-tube, allow the urine to flow gently upon the acid, so that the two liquids do not mix.
4. Stand the tube in the rack, and examine against a dark background.
5. If albumen is present, a sharply-defined white ring will be seen at the junction of the liquids.

Note.—*If only a trace of albumen is present, the white ring will not appear at once, and the tube must be allowed to stand aside for* 5 *to* 10 *mins.*, not longer.

Sources of error.—(*a*) *From presence of resinous acid, e.g., after ingestion of copaiba ;* (*b*) *Urea nitrate, only with concentrated urine and after standing aside some time ;* (*c*) *Uric acid, only with concentrated urine : the ring is somewhat above the junction of the two liquids.*

### B.—Heat and Acetic Acid.

1. Filter the urine if turbid.
2. Take a test-tube two-thirds full of the urine.
3. Boil the upper one-third of the liquid.
4. Add 2 or 3 drops of acetic acid.

5. If a precipitate is present after adding the acid, the urine in all probability contains albumen.

NOTE.—*In urines which contain only a trace of albumen, always examine the upper layer of the liquid in the test-tube against a dark background in a good light.*

## 31.—ESTIMATION OF ALBUMEN.

APPARATUS AND REAGENTS.—Esbach's albuminometer, picric acid solution (xix.), one dozen test-tubes, concentrated nitric acid, two graduated burettes, pipette.

### A.—ESBACH'S METHOD.

1. Fill the albuminometer up to the mark U on the tube.
2. Add the test solution to mark R.
3. Insert the stopper, and *gently* invert the tube several times.
4. Stand aside for 24 hours in an upright position, and then read off the amount of precipitate formed, from the graduated scale on the sides of the tube. This is so graduated as to express approximately the number of grammes of albumen per litre of urine.

NOTE.—*The urine should be fresh, acid, and of a low specific gravity, and, to obtain the latter, the urine may be diluted with water if necessary.*

*If more than four grammes of albumen per litre are present, the process should be repeated with diluted urine.*

*If the urine is diluted, the result must, of course, be corrected by multiplying this result by the number expressing the proportionate degree of dilution.*

## B.—Roberts' Method.*

1. Filter the urine if turbid.
2. Fill a graduated burette with urine, and another with water.
3. Prepare a series of test-tubes containing diluted urine, as follows:—

| No. | | | | Per cent. |
|---|---|---|---|---|
| 1. | 1 part urine to | 9 parts water | = 1 in 10 | = 0·033 |
| 2. | ,, ,, | 19 ,, ,, | = 1 ,, 20 | = 0·067 |
| 3. | ,, ,, | 29 ,, ,, | = 1 ,, 30 | = 0·100 |
| 4. | ,, ,, | 49 ,, ,, | = 1 ,, 50 | = 0·167 |
| 5. | ,, ,, | 79 ,, ,, | = 1 ,, 80 | = 0·267 |
| 6. | ,, ,, | 99 ,, ,, | = 1 ,, 100 | = 0·333 |
| 7. | ,, ,, | 149 ,, ,, | = 1 ,, 150 | = 0·500 |
| 8. | ,, ,, | 199 ,, ,, | = 1 ,, 200 | = 0·667 |
| 9. | ,, ,, | 299 ,, ,, | = 1 ,, 300 | = 1·000 |
| 10. | ,, | 399 ,, ,, | = 1 ,, 400 | = 1·333 |
| 11. | ,, ,, | 499 ,, ,, | = 1 ,, 500 | = 1·666 |

4. With a pipette place 2 c.c. of concentrated nitric acid into another series of eleven test-tubes.
5. With a fresh pipette take up 2 c.c. of the urine diluted 1 in 500, and allow it to flow gently upon the surface of the nitric acid in one of the tubes in the second series. (30, A.)
6. Repeat this with each specimen of diluted urine in succession (from the lowest upwards) till a white ring appears at the junction of the two fluids.

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* The percentages in above table are taken from Tappeiner's *Clinical Diagnosis*, trans. by E. J. McWeeney.

7. The test-tube of diluted urine which gives a white ring *after an interval of between* 2 *and* 3 *minutes* is the one from which the percentage must be read by means of the above table.

NOTE.—*The above test depends upon the fact that in urine which contains* 1 *part albumen in* 30,000 *the appearance of the white ring is delayed about* 3 *minutes after contact with the nitric acid.*

## 32.—DETERMINATION OF SUGAR.

APPARATUS AND REAGENTS.—Test-tubes and rack, Bunsen burner, water-bath, Fehling's test solutions A and B (xxi.), phenylhydrazin hydrochlorate, sodium acetate.

### A.—FEHLING'S TEST.

1. Into a test-tube pour equal volumes, about 2 c.c. of each, of Fehling's reagent, A and B.
2. In a second tube take 5 c.c. of urine.
3. Boil both these tubes over a flame *together*, and when boiling remove both from the flame, and allow them to cool for 20 seconds.
4. Pour the heated urine into the test-tube containing Fehling's solution, and stand aside without shaking the tube.
5. If sugar is present, a yellowish-green sediment will appear. If more than 1% of sugar is present, a precipitate will fall within 5 to 6 minutes.

NOTE.—*If this test is carefully performed,* 0·25% *of sugar can be demonstrated.*

### B.—PHENYLHYDRAZIN TEST.

1. Pour 3 c.c. of urine into a test-tube, and add as much of the phenylhydrazin hydrochlorate as can be placed on a shilling, and as much sodium acetate as can be placed on a florin.
2. Boil for one hour in a water bath.
3. Stand aside till cold, and then examine sediment under a microscope.
4. If sugar is present, delicate needle-like crystals having a radiating arrangement and of yellow colour will be seen.

## 33.—ESTIMATION OF SUGAR.

### A.—FERMENTATION TEST.

APPARATUS AND REAGENTS.—Special flask, urinometer, thermometer, yeast, filter paper.

1. Wash 1 gramme of yeast.
2. Pour 150 c.c. of urine into the glass cylinder.
3. Note accurately the specific gravity and temperature of the urine.
4. Cork the cylinder tightly so that no air can escape, and adjust the bent glass tubing so that the end inside the cylinder is above the upper surface of the urine.
5. Pour into the opening at the top of the glass tubing some mercury so as to fill the two limbs to the upper elbow.
6. Stand apparatus aside in a warm place 24 to 48 hours.
7. Note the amount of $CO_2$ given off during the process of fermentation, and take again accurately the specific

gravity of the urine, *at the same temperature* as the first observation.

8. Each degree of specific gravity lost represents one grain of sugar per fluid ounce.

### B.—TITRATION WITH FEHLING'S SOLUTION.

APPARATUS AND REAGENTS.—Graduated burette and stand, porcelain capsule, sand-bath and tripod, Bunsen burner, tile, filter paper and funnel, small beaker, measure, test-tube, Fehling's solutions A and B (xxi.).

1. Measure carefully 5 c.c. each of Fehling's solutions A and B; mix in a test-tube.
2. Take 5 c.c. of the urine, and add 20 c.c. of water, mixing thoroughly.
3. Pour some of the diluted urine into a burette, and note carefully the reading of the scale.
4. Place the 10 c.c. of Fehling's solution in a porcelain capsule on a sand-bath, and bring the solution to boiling point over a flame.
5. Remove the flame, and allow the dilute urine to run slowly into the heated solution, stirring with a glass rod.
6. Cease adding the urine the moment the blue colour disappears from the solution, and again bring it to boiling point.
7. This must be repeated till the blue colour no longer returns on boiling.
8. Filter a little of the heated solution through a moist filter paper; if the filtrate shows a blue colour it must be returned to the porcelain capsule, and after the addition of a little more urine be again brought to boiling point, and again filtered. *This must be repeated till the filtrate is colourless.*

9. When this occurs, acidulate the filtrate with acetic acid, and add a drop of potassium ferrocyanide; a brown coloration indicates that some of the copper is still unreduced, and another drop of the urine must be added to the heated solution. The most difficult part of the whole process is to determine the exact moment when all the copper is reduced, so as not to add any of the dilute urine after this point.

10. To calculate the amount of sugar present in the sample of urine used, proceed as follows: Read from the scale on the burette the amount of diluted urine used, and divide this by 5 to give the amount of undiluted urine used in the experiment.

    10 c.c. of the Fehling's solution prepared according to the formulæ given is completely reduced by 0·05 gramme of sugar, and this is therefore the quantity contained in the amount of urine used.

    From these data it is easy to calculate the quantity of sugar in grammes per litre, or in grains per fluid ounce (15·5 grains = 1 gramme).

## 34.—THE ESTIMATION OF URIC ACID (HOPKINS' METHOD).

Apparatus and Reagents.—Beakers, filter and funnel, glass rod and burette, solid ammonium chloride, ammonium hydrate, strong hydrochloric acid, sodium carbonate, twentieth normal permanganate solution, strong sulphuric acid.

1. To 100 c.c. of urine add 25 grammes of solid ammonium chloride.
2. Dissolve, and add sufficient ammonium hydrate to render the mixture alkaline.
3. Stir thoroughly till precipitate tends to settle, then stand aside till supernatant liquid is nearly clear.

4. Filter through a *small* filter paper, and wash with saturated solution of ammonium chloride; two washings are sufficient.
5. Wash precipitate of ammonium urate off the filter into a small beaker with a jet of hot distilled water.
6. Add 4 or 5 drops of strong hydrochloric acid, and heat till the liquid just boils.
7. Allow to stand over-night.
8. Filter off the crystals of uric acid through a *small* filter paper, *and measure the amount of mother liquor which comes through.*
9. Now wash the crystals with cold distilled water; *the greater part may be left in the beaker, and washed by decantation.*
10. Finally wash such crystals as are on the filter back into the beaker, add a small quantity of sodium carbonate, and heat till all the uric acid is dissolved.
11. Make the solution up to 100 c.c., and cool under tap.
12. Fill a burette with twentieth-normal-permanganate solution.
13. Add to the cooled solution of uric acid 20 c.c. of strong sulphuric acid, and immediately titrate with the permanganate solution.
14. The end point is given by the first appearance of a momentary but diffuse flush of colour throughout the liquid.
15. Each 1 c.c. of permanganate solution used is equal to 0·00375 gramme of uric acid. To the figures so obtained add 1 milligramme for each 15 c.c. of the mother liquor from which the uric acid was obtained (*see* 8 *above*).

## 35.—ESTIMATION OF UREA (GERRAD'S UREOMETER).

Apparatus and Reagents.—Gerrad's ureometer, hypobromite solution (xxxii.).

1. Measure 25 c.c. of the hypobromite solution into the flask.
2. Measure 5 c.c. of the urine into the small glass tube, and place it into the flask, being careful not to spill any of the urine into the hypobromite solution.
3. Fix the stopper tightly in the flask.
4. Open the clip of the graduated cylinder; pour water into the reservoir, and raise it until the water inside the cylinder is exactly at zero and on same level as that in reservoir; then close clip.
5. Slowly tilt the flask, and allow the urine and hypobromite solution to mix thoroughly.
6. Stand aside for 10 minutes to allow the mixture to cool.
7. Lower the reservoir till the water in cylinder and reservoir is at the same level.
8. Read the scale at the water level; the scale will give the percentage of urea present in the specimen of urine.
9. To obtain the number of grains per ounce, multiply the number on the scale by 4·375.

Note.—*It is better to use sealed tubes containing bromine, and to place 25 c.c. of soda solution with one of these in the flask. The bromine tubes will readily break if dropped to the bottom of the flask.*

## 36.—EXAMINATION OF URINE FOR BLOOD.

Apparatus and Reagents.—Ozonic ether, tincture of guaiacum, test-tube. 30 % acetic acid, solution of sodium-tungstate (xlvii.), solution of ammonia.

### A.—Colour.

Note colour of urine: varies from red to black; may be opaque or clear.

### B.—MICROSCOPE.

Centrifugalise, and examine sediment for red blood corpuscles, leucocytes, casts, bilharzia. (41.)

### C.—GUAIACUM TEST.

1. Take 1 c.c. each of tincture of guaiacum and ozonic ether in a test-tube, and mix.
2. Add an equal volume of the urine, holding the test-tube in a slanting position, so that the emulsion of guaiacum and ether rises to the top.
3. At the junction of the two liquids a blue ring will be seen if blood is present in any quantity.

### D.—SONNENSCHEIN'S TEST.

1. Add to the urine an excess of 30% acetic acid, and then precipitate with a solution of sodium tungstate (or precipitate the urine with a solution consisting of sodium tungstate, 30 parts; 30% acetic acid, 75 parts; water, 120 parts).
2. Filter, and dissolve the dirty rose-coloured residue in moderately dilute ammonia.
3. Spectroscope the solution: it gives the spectrum of alkaline methæmoglobin (three bands between C and E, yellow and green).

*This test is very sensitive and easy, and reacts with mere traces of blood.*

## 37.—TEST FOR BILE.

APPARATUS AND REAGENTS.—Beaker, flat dish, pipette, chloroform, 10% barium chloride solution, yellow nitric acid, tincture of iodine.

1. To 50 c.c. of urine add 5 c.c. of 10% solution of barium chloride and 5 c.c. of chloroform.

2. Stand aside for 15 minutes. A precipitate of phosphates and the chloroform will fall, carrying down the bile also if present.
3. Remove the chloroform and precipitate with a pipette.
4. Place in a flat porcelain dish, and allow the chloroform to evaporate.
5. A precipitate will remain.
6. Place a drop of yellow nitric acid on one portion of this precipitate, and a play of colours will appear around the drop if bile is present.
7. Upon another part of the precipitate place a drop of tincture of iodine; a green colour shows the presence of bile.

## 38.—EXAMINATION OF URINE FOR PUS.

Apparatus.—Test-tube, liquor potassæ, tincture of guaiacum.

### A.—Liquor Potassæ Test.

1. Take some of the deposit in a test-tube, and add liquor potassæ; a ropy gelatinous mass will form, to see which, pour from one test-tube into another.

### B.—Tincture of Guaiacum Test.

1. Acidulate the urine with acetic acid, and pass through a thick filter.
2. Treat the deposit on the filter with a little tincture of guaiacum; if pus is present, a deep blue tint appears.

### C.—Microscopic Examination.

See (17, C) and (23).

## 39.—CERTAIN DRUG REACTIONS IN URINE.

Apparatus and Reagents.—Test-tubes, spectroscope, chlorine water, chloroform, perchloride of iron, sodium hydrate, ammonium oxalate, magnesium wire, iodine, nitric acid, hydrochloric acid.

1. *Iodides in Urine.*

Take a specimen of the urine in a test-tube, add a few drops of chlorine water, which sets free the iodine.

On shaking with a little chloroform, the iodine is extracted by the chloroform, to which it imparts a violet colour.

(The Guaiacum Test often gives a blue or green colour.)

2. *Salicylic Acid.*

Add about 1 c.c. of the urine to 100 c.c. of water in a flask, allow a few drops of a solution of perchloride of iron to fall into the liquid.

If salicylic acid be present, the path of the drops will be marked by a rich purple colour.

If undiluted urine is used, the reaction is apt to be masked by the colourless phosphate precipitate which forms.

3. *Santonin.*

Notice the rich yellow colour of the urine.

Place some in a test-tube, and add sodium or potassium hydrate, when the liquid will assume a brilliant pink colour, showing the presence of santonin.

4. *Rhubarb and Senna.*

The urine when similarly treated becomes much darker, and if the phosphate precipitate be filtered off, it will be found to have a purple colour.

Acids change this purple to yellow.

5. *Lead.*

Add 1 grm. of ammonium oxalate to 150 c.c. of the urine.

Then place a clean and bright strip of pure magnesium in the urine.

After 24 hours examine the magnesium, wash it in distilled water, and dry it.

Then (*a*) warm the strip with a small crystal of iodine: this produces iodide of lead;

(*b*) or dissolve in $HNO_3$, and apply ordinary test for lead.

This test is very delicate.

6. *Copaiba.*

Add to the urine a little hydrochloric acid: it turns pink or red (if turbid add alcohol).

Now examine it spectroscopically: it shows three bands (one in the orange, one between D and E, and one between F and G).

## 40.—EXAMINATION OF URINE FOR TUBERCLE BACILLI.

Apparatus.—Centrifugalising machine, egg albumen, and apparatus as in (**17**).

1. Centrifugalise the urine thoroughly.
2. Take clean cover slips, and smear on one side of each a little egg albumen.
3. With a pipette remove a little of the sediment from the centrifugalised urine, and place a drop on the prepared surface of each cover slip.
4. Make films. (17.)
5. Dry in the air, and stain as usual. (17.)

## 41.—EXAMINATION OF URINARY SEDIMENTS.

Centrifugalise the urine, and examine the sediment microscopically, using a narrow diaphragm, concave mirror, and no condenser.

### A.—Organized Sediment.

1. Epithelium. (Pl. IV., A.)
2. Leucocytes (pus corpuscles). (Pl. IV., B.)
3. Red blood corpuscles. (Pl. IV., B.)
4. Casts. (Pl. III.)
5. New growths: carcinoma, &c.
6. Spermatozoa.
7. Bacteria.
8. Infusoria, &c.

A little iodine solution run under the cover glass will aid the observer in differentiating the various structures.

### B.—Casts.

(*a*) *Microscopic Examination.*

1. Centrifugalise the urine thoroughly.
2. With a pipette place a little of sediment upon a glass slide under a cover slip.
3. Examine the specimen microscopically:
    - (*a*) Cellular casts: (i.) renal epithelium. (Pl. III., A.)
        - (ii.) red corpuscles. (Pl. III., C.)
        - (iii.) shadows of red corpuscles.
        - (iv.) leucocytes. (Pl. III., B.)
    - (*b*) Granular casts: (i.) finely granular. (Pl. III., F.)
        - (ii.) coarsely granular. Pl. III., F.)
        - (iii.) with adherent epithelial cells.
        - (iv.) with adherent leucocytes. (Pl. III., B.)
        - (v.) with fat globules. (Pl. III., E.)
    - (*c*) Blood casts: granular and reddish brown.

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(*d*) Colloid casts: (i.) homogeneous. (Pl. III., D.)
(ii.) adherent cells, crystals, bacteria. (Pl. III., D.)

(*e*) Hyaline casts: very delicate and transparent; (Pl. III., D.)
adherent cells, &c. (Pl. III., D.)

(*b*) *Chemical Reactions.*

1. Allow some of the sediment to stand with Gram's iodine solution.
2. Allow another portion to stand with weak gentian violet.
3. Allow a third portion to stand with Marchi's fluid.
4. Next morning centrifugalise each, and wash sediment with water.
5. Then examine microscopically as above.

NOTE.—*The hyaline casts are stained by iodine or gentian violet; fat globules are stained black by Marchi's fluid.*

*As in the previous case* (41, A), *a little iodine solution run under the cover glass is a very great assistance.*

### C.—NON-ORGANIZED SEDIMENT.

### I. URINE ACID.

(*a*) *Amorphous Sediment.*

1. Granular: Urates (often together with crystals of uric acid or oxalate of lime). (Plate IV., C.)

   Test: Heat = sediment clears up.

2. Dumb-bells: (a) Calcium oxalate. (Pl. V., A.)

   Tests: insoluble in strong acetic acid; soluble in strong hydrochloric acid.

   (b) Calcium sulphate (rare). (Pl. V., F.)

   Test: insoluble in strong hydrochloric acid.

3. Highly refractive droplets: Fat. (Pl. III., E.)

   Test: soluble in ether.

NOTE.—*Granular urates somewhat resemble leucocytes, but with care this mistake should not be made.*

4. Yellow granules: Bilirubin (rare).

   Test for bile pigments.

(*b*) *Crystalline Sediments.*

1. Yellow large crystals of characteristic shape: Uric acid. (Pl. V., B.)

   Test: soluble in NaOH, reprecipitated by HCl.

2. Yellow small rhomboidal platelets: Bilirubin (often associated with yellow amorphous granules) (rare).

   Test for bile pigments.

3. Highly refractive octahedra: Calcium oxalate (envelope shaped). (Pl. V., A.)

   Tests: insoluble in strong acetic acid, soluble in strong HCl.

4. Crystals similar to (3): Triple phosphate. (Pl. IV., E.)

   Test: soluble in acetic acid.

5. Coffin-lid crystals: Triple phosphate (if urine is very slightly acid only).

6. Hexagonal plates: Cystin (rare). (Pl. V., E.)

7. Large, flat, refractive, long, rhomboidal plates: Normal phosphate magnesium (rare).

   Test: soluble in acetic acid.

8. Bundles of delicate needles: Tyrosin (rare). (Pl. V., C.)

   Tests: insoluble in acetic acid; soluble in $NH_4OH$ and HCl.

9. Wedge-shaped prisms, often in clusters: Neutral calcium phosphate (stellar phosphates). (Pl. IV., F.)

   Test: soluble in acetic acid.

### II. URINE ALKALINE.

(*a*) *Amorphous Sediment.*

1. Small granules (together with triple phosphate):

   (a) Earthy phosphates.

   Test: soluble in acetic acid without gas formation.

   (b) Calcium carbonate (rare).

   Test: soluble in acetic acid with gas formation.

2. Dumb-bells or spherical: Calcium carbonate (rare).
3. Large spiky spheres: Urate of ammonium. (Pl. V., D.) Test: soluble in acetic acid with formation of uric acid.

(*b*) *Crystalline Sediment.*

1. Coffin-lids: Triple phosphate.
2. Blue needles or tablets: Indigo (rare).

# POST-MORTEM ROOM METHODS.

## 42.— BACTERIOLOGICAL EXAMINATION OF TISSUES IN THE POST-MORTEM ROOM.

APPARATUS.—Bunsen burner, stout glass rod, thick platinum needle forceps, scissors, capillary pipettes, agar-agar tubes.

### A.—EXAMINATION OF THE BLOOD.

1. Open the thorax in the usual way.
2. With a pair of sterilised scissors lay open the pericardial sac.
3. Take a sealed capillary pipette, pass it through the flame, and with sterilised forceps break off the ends.
4. Hold the wall of the right auricle with a pair of sterilised forceps, and plunge one end of the pipette into the auricular cavity.
5. The blood will flow readily into the pipette as soon as the end enters the cavity of the auricle; obtain as much as is required, and withdraw the pipette.
6. Take 3 slanted agar-agar tubes, and holding them between the thumb and index finger, remove the cotton-wool plugs.

7. Allow a little blood to flow from the pipette on to the surface of the medium in each tube, replace the cotton-wool plugs, and seal the end of the capillary pipette to preserve the remaining blood for future examination if necessary.
8. Incubate the agar-agar tubes for 24 hours, and then examine any colonies that may be present. (25.)

NOTE.—*Any cavity containing liquid may be examined for micro-organisms by the above methods; but if many organisms are present, it is better to obtain a little of the liquid in a pipette as in* A, *and to place one drop in a broth-tube, then, after shaking, to inoculate three agar-agar tubes with one loop of this suspension.*

### B.—EXAMINATION OF THE SOLID TISSUES.

1. Expose the tissue to be examined, taking care to handle it as little as possible.
2. Take the stout glass rod, and heat it to a dull red heat in a Bunsen flame.
3. Cauterise carefully an area of about 1 in. square on the surface of the tissues to be examined.
4. Sterilise a stout platinum needle in the flame, and, *when it is cool*, thrust it into the tissue near the centre of the cauterised area.
5. Rotate the needle so that the end comes well into contact with the tissue under examination.
6. Withdraw the needle, and streak three agar-agar tubes in succession with the same needle, and without re-charging it.
7. In the same way inoculate broth and gelatine tubes.
8. Incubate for 24 hours, and then examine any colonies that may have grown. (25.)

*Another method which gives good results is to carefully cauterise the surface of the tissue, and then with sterilised scissors and forceps cut away a small piece of tissue below the cauterised surface, and place it in the medium.*

NOTE.—*The tubes should be kept for some days, and examined daily, as a growth often appears several days after inoculation.*

## 43. — EXAMINATION OF TISSUES IN POST-MORTEM ROOM.

APPARATUS.—2 % solution of potassium ferrocyanide, 1 % solution of hydrochloric acid.

### A.—For Free Iron.

1. Make an incision into the organ to be examined, and tear apart the two pieces thus divided.
2. Pour on to the torn surface a solution of potassium ferrocyanide, and allow it to remain 5 minutes.
3. Pour on a dilute solution of hydrochloric acid.
4. A blue tint is developed in those parts of the tissue which contain free iron.

NOTE.—*For microscopic examination see* (63).

### B.—For Amyloid Degeneration.

1. Make a section of the tissue to be examined, and wash away any blood with a stream of water.
2. Pour on to cut surface a solution of iodine, allow to remain 2 or 3 minutes.
3. Wash off the iodine with a stream of water.
4. If amyloid degeneration is present, the diseased portions of the tissue will be stained a deep mahogany brown colour.

NOTE.—*For microscopic examination see* (58).

## 44.—PRESERVATION OF MUSEUM SPECIMENS WITH FORMALIN.

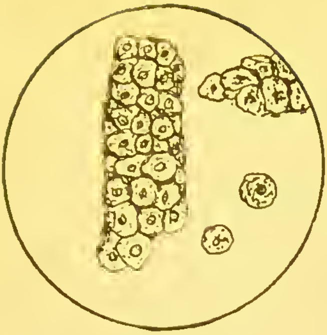
1. Take the tissue or organ to be preserved, and place it in three times its volume of the formalin solution (xxiii.) for 3 to 10 days, according to its density.
2. Remove the specimen to twice its volume of strong methylated spirit for 1 to 8 hours, till the specimen regains its natural colour.
3. Place it in the glycerine solution (xxviii.) for 2 weeks, then change to fresh glycerine solution; the specimen is now ready for mounting, or it may be kept unmounted in the glycerine solution for an indefinite period.

NOTE.—(*a*) *So far as possible, all dissection should be carried out before placing the specimen in formalin; but, if preferred, it may be hardened for 3 or 4 days first, and then the necessary dissection made, but care must be taken to return the specimen to the formalin solution for another 3 or 4 days before passing it on through spirit to glycerine solution.*

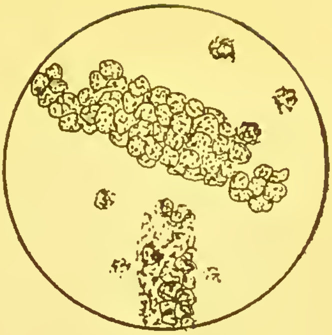
(*b*) *With organs such as lung, kidney, liver, spleen, better results will be obtained if these are injected through a large blood vessel with the formalin solution, and hardened a few days before a section is made, they should then be returned to the formalin for 3 or 4 days after making the section.*

(*c*) *The specimens should be carefully placed in the solutions in such a way as to preserve their natural shape; this is best done by supporting them lightly with cotton-wool.*

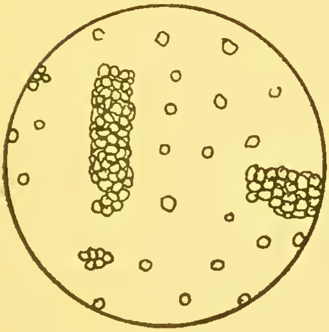
# PLATE III.



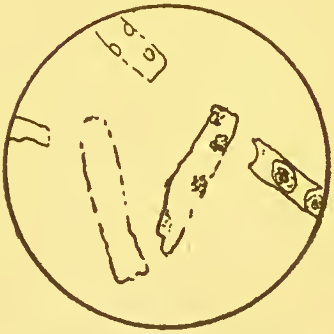
A. Epithelial casts.



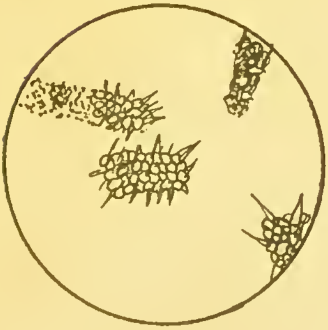
B. Cast of leucocytes.



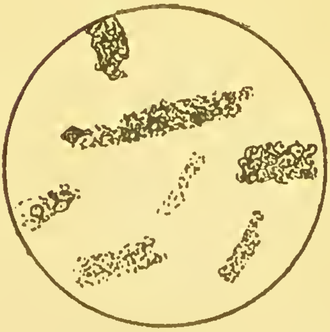
C. Blood casts.



D. Colloid and hyaline casts.

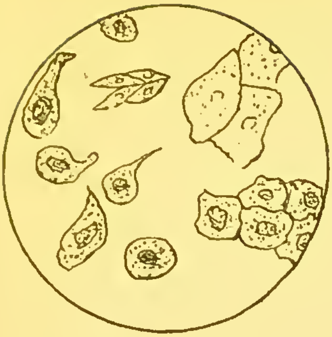


E. Fatty casts.

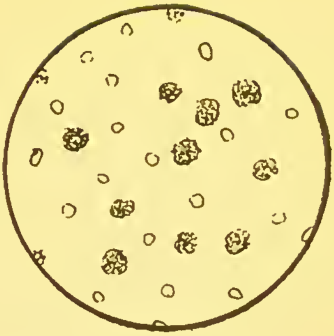


F. Granular casts.

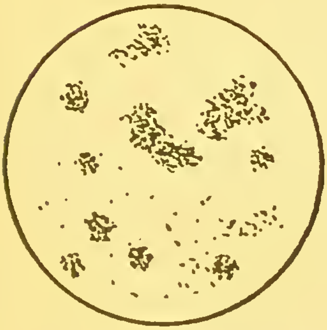
# PLATE IV.



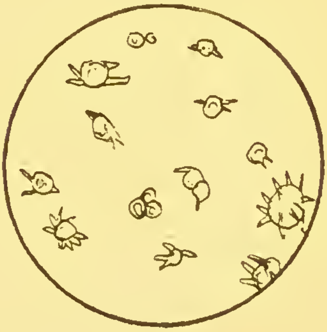
**A.** Epithelium.



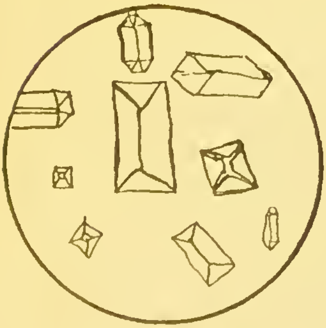
**B.** Leucocytes and red blood corpuscles.



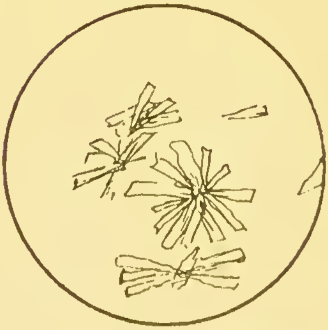
**C.** Granular urates.



**D.** Urate of ammonium.

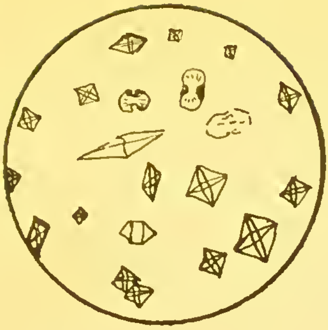


**E.** Triple phosphate.

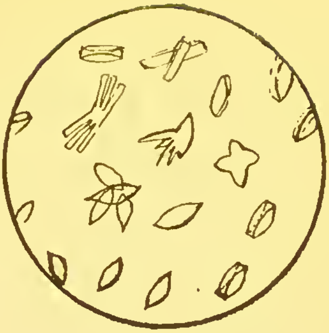


**F.** Neutral calcium phosphate.

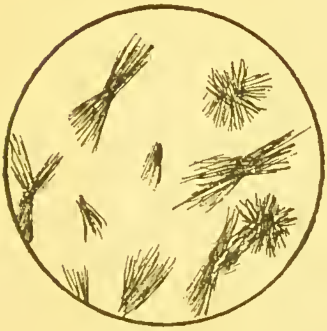
# PLATE V.



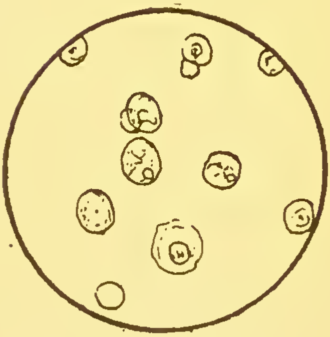
A. Calcium oxalate.



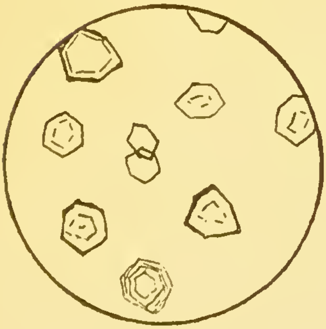
B. Uric acid.



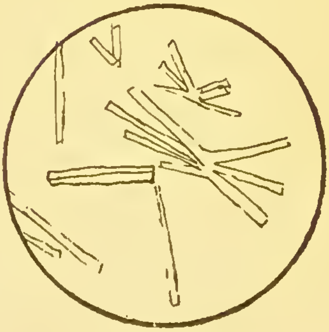
C. Tyrosin.



D Leucin.



E. Cystin;



F. Calcium sulphate.

# PRACTICAL MORBID HISTOLOGY.

## 45.—FIXING AND HARDENING TISSUES.

Apparatus, &c.—Wide-mouthed stoppered bottles, formol-Müller solution (xxiv.), Müller's fluid (xxxix.), sharp scalpel, forceps, labels.

1. Cut the tissues to be hardened into pieces about ½ in. cube.
2. Place 4 or 5 of these pieces into a 2 oz. stoppered bottle.
3. Fill up the bottle with formol-Müller solution.
4. Label carefully and neatly.
5. Within the next 24 hours pour off the formol-Müller solution, and refill bottle with some of the stock solution.
6. Repeat this within the next 48 hours.
7. At the end of the first week replace the formol-Müller solution with Müller's fluid.
8. Most tissues will be hardened sufficiently for examination at the end of a fortnight.

Note.—(*a*) *The above method for hardening is suitable for routine work, but for special researches other methods will be required.*

(*b*) *Always place the tissue in the hardening solution as soon as possible, in order that it may be fresh.*

(*c*) *The size and shape of the piece cut varies with the tissues to be examined; e.g., liver, kidney, spleen, should always be cut so as to include a portion of the capsule. Intestine, skin, and mucous membrane should be stretched on a piece of stout cardboard before they are hardened.*

(*d*) *Nervous tissues should be allowed to remain longer in the hardening solution than other tissues.*

(*e*) *Tissues may be hardened more rapidly by keeping them in a warm place (e.g., incubator) for a few days, or by adding a little more formalin to the hardening solution.*

(*f*) *The label should always give the date, patient's name and age, reference number to the Notes on the case, a short statement as to the nature of the tissue, and any other information that may be required.*

## 46.—FIXING TISSUES WITH BOILING WATER (=HEAT).

Apparatus, &c.—Beaker of boiling water, Bunsen burner, sand-bath and tripod, sharp scalpel, forceps, solution of gum arabic (xxx.).

1. Have ready a small beaker of actively boiling water.
2. Cut a piece of the tissues to be examined about $\frac{3}{4}$ in. cube.
3. Plunge it into the boiling water for 3 mins. to 5 mins., keeping the water at boiling point the whole time.
4. Remove the tissue from the beaker and allow it to cool in the air.
5. Place in a solution of gum arabic till ready to cut.
6. Cut sections on Williams' or other freezing microtome. (50.)
7. Stain with alum carmine. (53.)

Note.—(*a*) *The tissue may be embedded in paraffin* (48) *or celloidin* (49).

(*b*) *This method is particularly well adapted for the rapid examination of organs in the post-mortem room, and also when it is desired to fix œdematous tissues.*

(*c*) *The sections may be frozen and cut immediately after boiling.*

## 47.—DECALCIFICATION OF TISSUES.

Apparatus, &c.—Four-oz. large wide-mouthed stoppered bottles, Von Ebner's HCl solution (l.), strong knife, scissors, small saw, labels, formol-Müller solution (xxiv.), Müller's fluid (xxxix.).

1. Cut and harden the tissues as usual for a fortnight. (45.)
2. Wash well in water for 12 hours.
3. Replace the tissue in the bottle, and fill up with Von Ebner's solution.

4. Change the decalcifying liquid every other day.
5. Keep the tissue in the fluid until it cuts readily with a knife; if the tissue is properly decalcified, there will be no gritty sensation on section.
6. When decalcification is complete, wash the tissue in water for 24 hours.
7. Transfer to Müller's fluid for one week.
8. The specimen is now ready for examination.

NOTE.—*Other re-agents may be used as decalcifying solutions, but the above will serve for all ordinary work.*

## 48.—EMBEDDING TISSUES IN PARAFFIN.

APPARATUS, &c.—Nine 2-oz. wide-mouthed stoppered bottles, four containing 25°/o, 50°/o, 75°/o and strong methylated spirit respectively; two filled with absolute alcohol, labelled No. 1 and No. 2; three filled with cedar oil, and labelled No. 1, No. 2, No. 3; hard paraffin of melting point 56° C., paraffin bath, moulds, sharp knife, forceps, wooden cubes 1″ square.

1. With a sharp knife remove a piece of the hardened tissue (XLV.), *taking care not to cut it more than $\frac{1}{3}$ in. thick, and to note carefully the surface from which the section is to be made.*
2. Wash the tissue in water for 12 hours.
3. Place the tissue in 25% spirit for 12 hours.
4. „ „ 50% „ „
5. „ „ 75% „ „
6. „ „ strong „ „
7. „ „ abs. alcohol (No. 1) for 12 hours.
8. „ „ „ (No. 2) „ „

9. Place the tissue in cedar oil (No. 1) for 12 hours.
10. „ „ „ (No. 2) „ „
11. „ „ „ for 24 hours.
12. „ „ „ (No. 3) till clear.
13. Drain off excess of oil, and put the tissue into the paraffin bath for 12 hours.
14. Transfer the tissue to another bath of fresh paraffin and leave for 12 hours.
15. Place the tissue in a third bath of paraffin for another 12 to 24 hours.
16. Pour into a mould some melted paraffin from the last bath, and with a pair of warm forceps place the tissue (*with the surface to be cut downwards*) in the mould, fill up the mould with melted paraffin, and set aside to cool.
17. When the paraffin begins to solidify, put the mould containing it into cold water until the wax is quite solid.
18. Remove the paraffin from the mould, and carefully trim away excess of paraffin, being careful to remember which side of the embedded tissue you wish to cut, namely, that at the bottom of the mould.
19. Take one of the small wooden cubes, and with warm forceps melt the surface of the block of paraffin, and press it on to the wooden block; it should remain firmly fixed when set.
20. The tissue is now ready to be cut. It should be placed in a large pill-box, to the lid of which a careful copy of the label on the bottle should be affixed.

## 49.—EMBEDDING TISSUES IN CELLOIDIN.

Apparatus, &c.—Ten 1-oz. wide-mouthed stoppered bottles, four containing 25°/₀, 50°/₀, 75°/₀ of strong methylated spirit respectively; three containing absolute alcohol and labelled Nos. 1, 2, 3; one containing a mixture of absolute alcohol and ether, equal parts; one containing thin celloidin solution (xiv.), and one containing a thick solution of celloidin (xiv.); sharp scalpel, forceps, beaker, wooden cubes.

1. Harden the tissue as usual. (**45.**)
2. With a sharp knife cut off the piece of tissue to be examined: this should be about ¼ in. thick.
3. Wash in water for 12 hours.
4. Place in 25% spirit, 12 hours.
5. „ 50% „ „
6. „ 75% „ „
7. „ strong „ „
8. „ abs. alcohol (No. 1), 12 hours.
9. „ „ (No. 2), „
10. „ „ (No. 3), „
11. „ alcohol and ether, 6 hours.
12. „ thin solution of celloidin, 7 days.
13. „ thick „ „ 7 „
14. Place the specimen on a wooden cube, and pour round it some of the thick celloidin.
15. Dry very slowly under a bell-jar, watching the specimen carefully, and when the celloidin is so firm that the ball of the finger will only make a small depression, place the whole in a solution of 80% alcohol for 2 days.
16. The specimen is now ready for cutting.

Note.—*The above 'times' may be shortened in practice. The exact time depends on the size and kind of tissue to be embedded, and experience alone can teach this.*

## 50.—CUTTING SECTIONS ON A FREEZING MICROTOME (WILLIAMS').

Apparatus, &c.—Williams' ether freezing microtome (Fig. 4) with spray, bellows, razor carrier, sharp razor, ether in a small bottle, camel's-hair brushes, gum solution, forceps, glass needle, two large capsules.

1. Place the tissue in the gum solution, if possible, a few hours before it is to be cut.
2. Take a sharp razor, and fix it firmly and evenly in the carrier (Fig. 4, B).

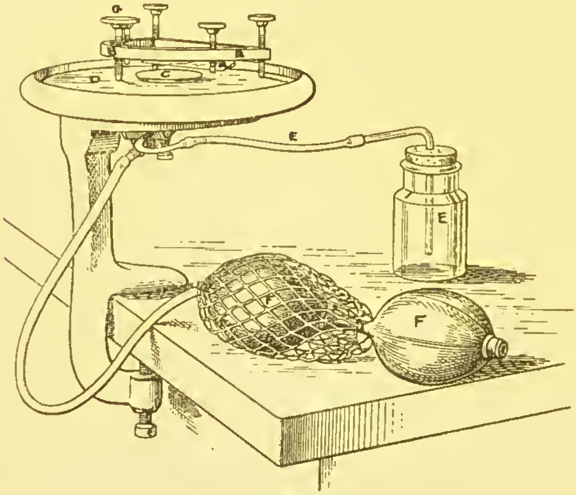


Fig. 4.

A, Razor; B, Razor carrier; C, Metal disc; D, Glass bed-plate; E, Ether spray; F, Rubber bellows; G, Micrometer screw.

3. Place the tissue on a little gum in the centre of the metal disc (Fig. 4, C).
4. Moisten the glass plate (Fig. 4, D) of the microtome with water.
5. Work the ether spray (Fig. 4, E, F) slowly, and, as the gum begins to freeze, paint a little more gum around the tissue.

6. Continue to spray until a sufficient thickness of tissue has been frozen, *e.g.*, about $\frac{1}{8}$ in.
7. Adjust the edge of the razor so that it is on a level with the surface of the frozen gum.
8. With a sliding cut remove the upper portion of the tissue (*i.e.*, the portion not frozen), and replace it in the gum solution.
9. Turn the micrometer screw (Fig. 4, G) about $\frac{1}{8}$ of a circumference, and, pressing the carrier firmly and evenly along the bed-plate, remove a thin section from the frozen mass.
10. Repeat this several times until a few sections have been cut.
11. Transfer the sections to a large capsule of water.
12. Select the thinnest and most perfect of these, and place them in the second capsule of water; they are now ready for staining.

## 51.—CUTTING SECTIONS ON A CAMBRIDGE ROCKING MICROTOME (Fig. 5).*

APPARATUS, &c.—Cambridge rocker, sharp razor, water bath (temp. 58° C.), camel's-hair brush, fine forceps, fixative solution, blotting-paper, small cardboard tray.

1. Adjust the razor firmly in its place in the microtome (Fig. 5, A).
2. Bring the arm of the rocker (Fig. 5, B) well back: this is done by turning the micrometer screw (Fig. 5, C) to the left.

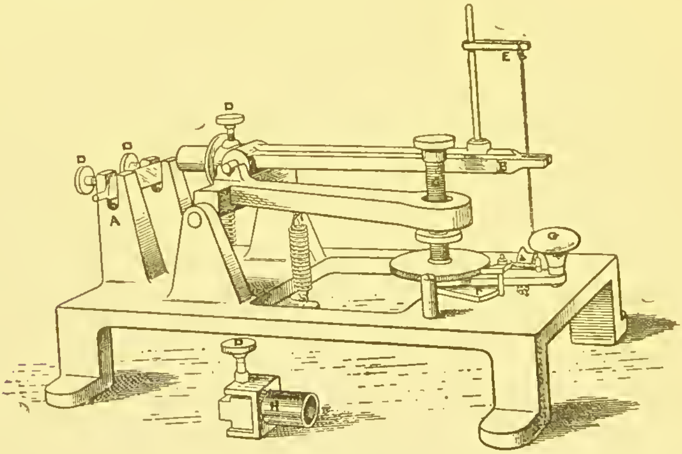


Fig. 5.

A, Razor holder; B, Arm; C, Micrometer screw; D, Clamp screws; E, String holder; F, Regulator; G, Handle; H, Specimen holder.

3. Fix the wooden block with the tissue attached (48) firmly on to the specimen holder (Fig. 5, H).
4. Screw the carrier firmly on to the arm of the microtome (Fig. 5, B) so that the surface of the paraffin block just misses the edge of the razor when the arm is lowered.
5. Adjust the arm of the microtome by lowering or raising the string-holder (Fig. 5, E) so that the upper edge of the paraffin block is just below the edge of the razor.

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* A new and improved pattern has recently been designed by the Cambridge Scientific Instrument Company.

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6. Adjust the regulator (Fig. 5, F) so that about 8 notches of the wheel are in use: this is done by bringing forward or pushing back the regulator.
7. Work the handle (Fig. 5, G) backwards and forwards, this will carry the arm of the microtome forwards, and thin sections will be obtained from the paraffin block.
8. When the embedded tissue is reached, it will be found that thin sections are cut at each to-and-fro movement of the handle; the thickness of these may be varied by moving the regulator so as to take in fewer or more notches; the more notches the wheel is carried round, the greater the thickness of the sections.
9. Get ready the water bath to required temperature, and proceed to cut your sections.
10. As each section comes off the specimen, remove it carefully with the forceps and camel's-hair brush on to the surface of the water in the bath; it will at once spread out as a thin film.
11. Take a clean cover-slip, and smear the smallest possible quantity of the fixative solution on to the surface; then with the forceps pass it carefully under the section, and with the camel's-hair brush manipulate the section so that it is taken from the water on to the centre of the cover-slip.
12. Drain off excess of water on a piece of blotting-paper.
13. Place the section in a cardboard tray.
14. When as many sections as are required have been mounted, place the tray in a warm dry place (*e.g.*, incubator) for 24 hours; the sections are then ready to be stained.
15. Replace the paraffin block in the pill-box.

## 52.—CUTTING SECTIONS ON A SLIDING MICROTOME. (Fig. 6).

Apparatus, &c.—Sliding microtome and sharp knife, camel's-hair brush, large glass dish, spirit.

1. Trim the mass of celloidin on the wooden cube, so that the tissue remains embedded in a square mass of the celloidin, and remains fixed to the wooden cube.
2. Fix the wooden cube firmly in its clamp (B), and adjust the clamp to its carrier (A), place the carrier on the inclined plane (C).

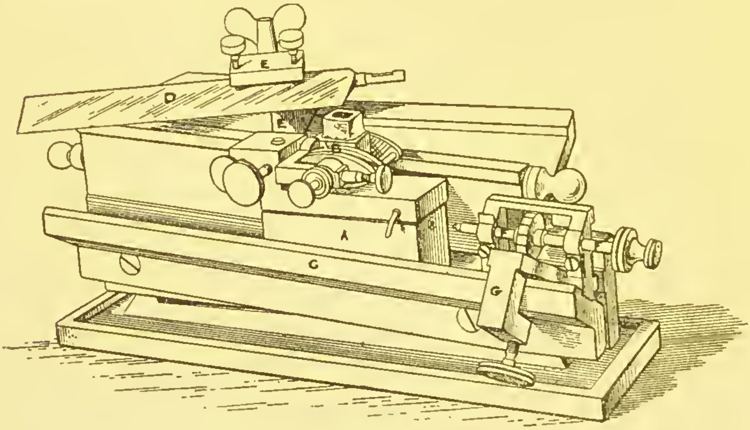

Fig. 6.

A, Specimen carrier; B, Clamp; C, Inclined plane; D, Knife; E, Knife carrier; F, Micrometer screw; G, Clamp of Micrometer.

3. Fix the knife in its carrier (E).
4. Arrange the knife and the specimen carrier (A) so that when the knife approaches the specimen the edge of the razor shall be in the same plane as the upper surface of the specimen.
5. Adjust the knife obliquely on its carrier (E) so that a great part of the length of the blade is used.

6. Set the micrometer screw (F) well back, and clamp it tightly on the inclined plane (C), with the end of the screw touching the specimen carrier.
7. Keep the knife and specimen moist with spirit during the whole process; the celloidin must never be allowed to dry.
8. Bring the knife with a steady pressure, evenly and firmly, towards you, and thus remove the upper portion of the celloidin block.
9. Remove this with the camel's-hair brush, and push back the knife.
10. Turn the micrometer screw, and then advance the section carrier along the inclined plane, so as to raise it sufficiently for a thin section to be taken off with the next movement of the knife.
11. Again moisten the knife and specimen with spirit.
12. Again bring the knife toward you, and remove a section.
13. With the camel's-hair pencil place this section in a large dish of water.
14. Repeat until you have made as many sections as you require.
15. They are now ready for staining. (56.)

NOTE.—*The cut sections may be kept in* 80% *spirit if not required at once for staining.*

## 53.—STAINING FROZEN SECTIONS WITH ALUM CARMINE.

APPARATUS, &c.—Glass capsules, watch-glasses, needles, forceps, blotting-paper, Canada balsam, slides, cover-slips, alum carmine (vi.), spirit, clove oil.

1. Filter a little stain into a watch-glass.
2. Place the section in this until stained, for 10 mins. to 1 hour.

3. Wash well in a capsule of water, 10 mins.
4. Wash again in water, 5 mins.
5. Place the section in a capsule of strong spirit for 2 mins.
6. Place the section in fresh strong spirit for 2 mins.
7. Remove the section to a watch-glass containing clove oil, 2 mins.
8. Place section on a glass slide with a section lifter, and drain off the clove oil by standing the slide on end for 5 mins.
9. Remove with blotting-paper any clove oil remaining around section.
10. Mount in Canada balsam.

## 54.—STAINING FROZEN SECTIONS WITH HÆMATEIN AND COUNTER-STAINING BY VAN GIESON'S METHOD.

APPARATUS as in **53**, with hæmatein (xxxi.) and Van Gieson's (xlix.) stain.

1. Filter a little hæmatein into a watch-glass.
2. Place the section in this for 2 mins.
3. Remove, and wash well in water for 5 mins.
4. Wash again in fresh water till quite blue, 1 to 5 mins.
5. Place the section in a watch-glass containing Van Gieson's stain for ½ min.
6. Wash the section in water till the fibrous tissue shows a bright pink colour.
7. Proceed as in 5 to 10 above.

## 55.—STAINING AND MOUNTING PARAFFIN SECTIONS.

APPARATUS, &c.—Covered capsules, one drop-bottle of absolute alcohol, one drop-bottle of xylol, Canada balsam, spirit, clearing fluid, hæmatein (xxxi.), Van Gieson's stain (xlix.), fine forceps, mounted needles, filter paper, blotting-paper, slides, labels.

1. Place the cover-glass, with section affixed, in xylol 1 min., *to dissolve out the paraffin.*
2. Wash in fresh xylol for 1 min., *to remove the last trace of paraffin.*
3. Wash in spirit 2 mins., *to remove excess of xylol.*
4. Wash in fresh spirit 1 min., *to remove the last trace of xylol.*
5. Wash in water for 5 mins., *to remove the spirit.*
6. Filter a little hæmatein into a watch-glass, and place the section in this for about 10 mins.
7. Drain off excess of stain, and wash well in water for 1 min.
8. Place the section in fresh water 5 mins., till blue.

   If the section is to be counter-stained, place it into Van Gieson's stain for ½ min.; wash quickly in water till fibrous tissue shows a bright pink colour.
9. Place the section into a capsule containing spirit for 2 mins., *to remove the water.*
10. Remove to fresh spirit, 2 mins., *to remove the last trace of water = dehydration.*
11. Wash well in clearing fluid, *to clear, and thus render transparent.*
12. Wash in xylol for 1 min., *to clear, and thus render transparent.*
13. Place in xylol for 2 mins., *to clear, and thus render transparent.*
14. Drain off excess of xylol, and mount in Canada balsam.
15. Label neatly and carefully.

## 56.—STAINING CELLOIDIN SECTIONS.

### A.—Ordinary Methods.

Apparatus, &c., as required for (**55**).

1. Float the section on to a clean glass slide.
2. Blot quite dry with several layers of blotting-paper.
3. Filter a little stain on to the section, and allow it to remain till sufficiently stained (about 10 mins.).
4. Wash in water.
5. If necessary, counter-stain as usual. (55.)
6. Again wash in water.
7. Wash with spirit.
8. Place in spirit, 2 mins.
9. Drop on the section a little carbol xylol.
10. Clear with xylol.
11. Mount in Canada balsam.

Note.—*Clove oil cannot be used, as it dissolves celloidin.*

### B.—Weigert's Method.

Apparatus, &c.—Solution of copper acetate (xvii.), Weigert's hæmatoxylin (lii.), differentiating solution (li.), carbol xylol, spirit xylol, Canada balsam, capsules, glass, needles, slides, and cover-slips.

1. Embed the tissue in celloidin. (49.)
2. Place the wooden cube with the embedded tissue into the copper acetate solution for 24 hours.
3. Cut sections on a sliding microtome (52), float them into water.
4. Stain with Weigert's hæmatoxylin for at least 2 hours.
5. Wash well in water.
6. Differentiate in the differentiating solution.
7. Wash well in water, and leave the section to wash for 12 hours.

8. Place in methylated spirit, 15 mins.
9. Place in a second capsule of methylated spirit, 10 mins.
10. Place in carbol xylol, 15 mins.
11. Place in xylol till clear.
12. Mount in Canada balsam.

## 57.—STAINING FROZEN SECTIONS FOR FATTY CHANGES.

### A.—With Marchi's Fluid.

Apparatus, &c.—Glass capsules, watch-glass, needles, forceps, slides and cover-slips, blotting-paper, labels, clove oil, Canada balsam, spirit, and the following solutions: Marchi's fluid (xxxvii.), pyrogallic acid (xlv.), oxalic acid (xliii.), potassium permanganate (xliv.).

1. Place the sections in Marchi's fluid for 24 hours.
2. Wash well in a large volume of water.
3. Wash again in fresh water.
4. Place the section in a solution of pyrogallic acid for 1 min.; it will turn black.
5. Wash well in a large volume of water.
6. Wash again in fresh water.
7. Place the section in a solution of potassium permanganate for 1 min.; it will turn brown,
8. Wash well in water.
9. Again wash in fresh water.
10. Place the section in a solution of oxalic acid for 5 mins.; it will become bleached.
11. Wash well in large volume of water for 5 mins.
12. Wash again in fresh water.
13. Stain the section with alum carmine for 12 to 24 hours.

14. Wash well in water for $\frac{1}{2}$ hour.
15. Wash again in fresh water.
16. Place in strong spirit for 5 mins.
17. Remove to fresh strong spirit for 5 mins.
18. Clear with clove oil for 2 mins.
19. Mount in Canada balsam.

### B.—Staining for Fatty Changes with Soudan III.

Apparatus.—Hæmatein solution (xxxi.), Soudan III. solution (xlvi.), 60% spirit, Farrant's solution (xx.), capsules, glass needles, blotting-paper, slides, and cover-slips.

1. Harden the tissues in formol-Müller. (45.)
2. Cut on freezing microtome. (50.)
3. Stain with hæmatein. (54.)
4. Wash well in tap water.
5. Stain with Soudan III. solution for 3 mins.
6. Wash in 60% spirit.
7. Mount on glass slides, and blot off excess of dilute spirit.
8. Mount in Canada balsam.

Note.—*If the tissues are very delicate, or if it is otherwise preferred, the staining may be carried out in bulk, by passing small pieces of the tissue hardened in Müller's or Marchi's fluid through the various solutions* 1–13, *and then embedding in paraffin, and counter-staining (after cutting) by any one of the usual methods.*

## 58.—STAINING SECTIONS FOR AMYLOID DEGENERATION.

Apparatus, &c.—Glass capsules, watch-glass, beaker, needles, forceps, blotting-paper, section lifter, slides and cover glasses, labels, solutions of methyl-violet (xxxviii.) and acetic acid 1%, Farrant's medium (xx.).

1. Place the section in a solution of methyl-violet for 10 mins.
2. Wash well in water.

3. Wash in a 1% solution of acetic acid.
4. Wash again in water.
5. Mount in Farrant's medium.

## 59.—STAINING PARAFFIN SECTIONS BY WEIGERT'S FIBRIN METHOD.

APPARATUS, &c, as for **(55)**, and in addition carbol-gentian-violet (xii.) and Weigert's iodine (liii.), aniline oil and xylol (v.).

1. Pass the section through xylol and spirit to water. (55.)
2. Stain in alum carmine for 1 to 12 hours. Eosin—
3. Wash well in water.
4. Stain with carbol-gentian-violet, 5 to 20 mins.
5. Blot off excess of stain.
6. Pour on to section a little Weigert's iodine, leaving it for ½ min.
7. Blot the section quite dry.
8. Pour on aniline oil and xylol till no more blue colour comes away, and section is quite clear.
9. Wash well in xylol.
10. Mount in Canada balsam.

NOTE.—(*a*) *Fibrin. Many micro-organisms and the colloid material in colloid carcinoma stain a deep blue. The tissue is counter-stained with alum carmine.*

(*b*) *Frozen sections and sections embedded in celloidin may also be stained by this method, after floating them on to a glass slide, and blotting them firmly on to it. The staining process is then carried out on the glass slide as above.*

## 60.—STAINING PARAFFIN SECTIONS FOR MICRO-ORGANISMS.

Apparatus, &c., as in (**55**), and in addition the special stains required: Löffler's methylene blue (xxxv.), Weigert's fibrin method (**59**), Gram's method (**25**, C), and carbol fuchsine (xi.).

### A.—Staining Sections with Löffler's Methylene Blue.

1. Remove paraffin by passing section through xylol and spirit to water. (55.)
2. Stain in Löffler's methylene blue, 5 mins.
3. Wash well in water.
4. Decolorise with spirit until sufficient stain has come away, and the histological structure of the tissue is well shown; *practice alone will enable the worker to know when this is accomplished.*
5. Blot quite dry.
6. Clear in xylol.
7. Mount in Canada balsam.

### B.—Staining by Weigert's Fibrin Method.

See (59).

### C.—Staining by Gram's Method.

1. Remove paraffin by passing section through xylol and spirit to water. (55.)
2. Stain with carbol-gentian-violet from 5 to 20 mins.
3. Pour on a little Gram's iodine.
4. Wash in water.
5. Decolorise in spirit till no more blue comes away.
6. Clear in xylol.
7. Mount in Canada balsam.
8. The specimen may be counter-stained with alum carmine, as in Weigert's fibrin method, if required.

### D.—Staining Tissue for Tubercle Bacilli.

1. Remove the paraffin by passing through xylol and spirit to water.
2. Place in warm carbol fuchsine for 5 hours.
3. Wash in water.
4. Dip the section into 25% HCl.
5. Decolorise in spirit.
6. Wash in water.
7. Stain in methylene blue as in A.
8. Blot quite dry.
9. Clear in xylol.
10. Mount in Canada balsam.

Note.—*Frozen or celloidin sections may be stained as above, if previously mounted on a glass slide as in* (56).

## 61.—TO STAIN ELASTIC TISSUE.

Apparatus, &c.—Bunsen burner, porcelain capsule, watch-glasses, slides and cover slips, spirit, clove oil, Canada balsam, Unna's orcein stain (xlii.).

1. Place the sections in a porcelain capsule with a little of the orcein stain.
2. Warm the porcelain capsule over a Bunsen flame to a temperature of about 30° C., until the alcohol in the stain evaporates and the stain becomes quite thick, about 10 to 20 minutes.
3. Wash the sections in a large volume of spirit for 5 minutes.
4. Place in fresh spirit for 2 minutes.
5. Clear in clove oil.
6. Mount in Canada balsam.

## 62.—STAINING SECTIONS OF NERVOUS TISSUE.

### A.—Heller's Method.

Apparatus, &c.—Glass capsules, slides, cover slips, glass needles, spirit, clove oil, Canada balsam, pyrogallic acid solution (xlv.), 2% solution of oxalic acid, 0·25% solution of potassium permanganate, Marchi's fluid (xxxvii.).

1. Place the sections in Marchi's fluid from 1 to 12 hours.
2. Wash thoroughly in water.
3. Transfer to pyrogallic acid solution till black.
4. Wash again in excess of water.
5. Place in 0·25% solution of potassium permanganate till yellow.
6. Wash in water.
7. Bleach in 1% solution of oxalic acid.
8. Again wash thoroughly in water.
9. Counter stain with alum carmine.
10. Wash well in water.
11. Dehydrate in spirit.
12. Clear in clove oil.
13. Mount in Canada balsam.

### B.—Nissl's Stain for Nerve Cells.

Apparatus, &c.—Watch-glasses, slides and cover slips, glass needles, Bunsen burner, blotting-paper, 96% alcohol, methylene blue solution (xli.), aniline alcohol (iii.), oleum cajuputi, benzene, benzene-colophonium (vii.).

1. Harden the tissue, *which must be quite fresh*, in 96% alcohol for 12 hours.
2. Transfer to fresh 96% alcohol for 12 hours.
3. Fix the tissue (*without embedding*) on a wooden cube with solution of gum arabic.
4. Place in absolute alcohol, 24 hours.
5. Cut on a sliding microtome. (52.)

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6. Stain with methylene blue solution in a watch-glass, heating slowly over a small flame till the first bubble is given off = about 65° C.
7. Differentiate in aniline alcohol until no more large clouds of stain come off.
8. Float on a slide, and blot quite dry.
9. Clear with oleum cajuputi.
10. Blot dry again.
11. Pour on benzene.
12. Mount quickly in benzene-colophonium, from which benzene gas has been driven by heating over a flame.

## 63.—MICROSCOPIC EXAMINATION OF TISSUES FOR FREE IRON.

1. Harden the tissue as usual. (45.)
2. Prepare frozen sections. (50.)
3. Place the sections in a 2 % solution of potassium ferrocyanide, 30 minutes.
4. Remove to 1 % solution of hydrochloric acid, 30 minutes.
5. Wash well in distilled water.
6. Stain with alum carmine 2 hours, dehydrate, clear, and mount. (53, A.)

## 64.—FORMULÆ.

### . Agar Medium.

To every litre of broth add 20 grammes of agar (fibre), which has been soaked in 1% acetic acid for ¼ hour, then well washed; boil till all the agar is dissolved, neutralise carefully; when cool enough add white of egg. Heat until it becomes clear, filter, sterilise on two successive days for ½ hour.

### ii. Alcoholic Eosine.

| | |
|---|---|
| Eosine ... ... ... | ·5 grammes. |
| Distilled water ... | 30 c.c. |
| Absolute alcohol ... | 70 ,, |

### iii. Aniline Alcohol.

| | |
|---|---|
| Aniline oil .. ... | 10 c.c. |
| Alcohol 96% ... ... | 90 ,, |

### iv. Aniline Gentian Violet.

90 c.c. of a 4% solution of aniline water (filtered).

10 c.c. of a saturated alcoholic sol. of gentian violet.

### v. Aniline Oil and Xylol.

| | |
|---|---|
| Aniline oil... ... ... | 2 parts. |
| Xylol ... ... ... | 1 part. |

### vi. Alum Carmine.

| | |
|---|---|
| Carmine ... ... ... | 5 grammes. |
| 5% solution of alum ... | 100 c.c. |

Boil for ½ hour, cool and filter.

### vii. Benzene-colophonium.

Dissolve colophonium in benzene, filter, and evaporate to required consistency.

### viii. Bismarck Brown.

Saturated aqueous solution of Bismarck brown. Boil and filter.

### ix. BICHROMATE SOLUTION.

| | |
|---|---|
| Sulphuric acid ... ... | 60 c.c. |
| Potassium bichromate ... | 60 grammes. |
| Distilled water ... ... | 1000 c.c. |

### x. BROTH MEDIUM.

Take 1 lb. of beef, trim off all fat, add 1 litre of water, boil for 10 minutes; let it cool, then filter; add to this 10 grammes of peptone and 5 grammes of salt. Steam in steriliser for ½ hour, neutralise carefully, heat for another ½ hour. Filter, sterilise on two successive days for ½ hour.

### xi. CARBOL FUCHSINE.

90 c.c. of a 5% solution of carbolic acid.

10 c.c. of a saturated alcoholic solution of fuchsine.

### xii. CARBOL GENTIAN VIOLET.

10 c.c. of a saturated alcoholic sol. of gentian violet.

100 c.c. of a 1% sol. of carbolic acid (abs. phenol).

### xiii. CARBOL XYLOL.

| | |
|---|---|
| Xylol... . ... ... | 75 c.c. |
| Carbolic acid crystals ... | 25 grammes. |

### xiv. CELLOIDIN SOLUTION (THIN).

| | |
|---|---|
| Perfectly dried celloidin ... | 4 parts by weight. |
| Absolute alcohol ... ... | 50 ,, ,, measure. |
| Ether ... ... ... | 50 ,, ,, ,, |

### CELLOIDIN SOLUTION (THICK).

| | |
|---|---|
| Perfectly dried celloidin ... | 8 parts by weight. |
| Absolute alcohol ... ... | 50 ,, ,, measure. |
| Ether ... ... ... | 50 ,, ,, ,, |

### xv. CITRATE SOLUTION.

| | |
|---|---|
| Citric acid | ½ gramme. |
| Sodium chloride | ½ ,, |
| Distilled water | 100 c.c. |

### xvi. CLEARING FLUID.

| | |
|---|---|
| Beechwood creosote | 100 c.c. |
| Pure turpentine | 400 ,, |

Mix thoroughly.

### xvii. COPPER ACETATE SOLUTION.

| | |
|---|---|
| Saturated sol. of copper acetate | 50 c.c. |
| Distilled water | 50 ,, |

### xviii. EHRLICH'S TRIACID STAIN.*

(Obtain the following pure dyes from the Analin Dye Company, of Berlin :—
Orange g., Acid fuchsin, Methyl green.
Make saturated watery solutions of the three dyes, and clear them by allowing then to stand some days. To prepare the stain, make the following mixture, using the same measuring glass, and from the addition of methylene green onwards keep the liquid thoroughly shaken.)

| | |
|---|---|
| Orange g. solution | 13·14 c.c. |
| Acid fuchsin solution | 6·7 ,, |
| Distilled water | 15 ,, |
| Alcohol | 15 ,, |
| Methyl green | 12·5 ,, |
| Alcohol | 10 ,, |
| Glycerine | 10 ,, |

The solution may be used at once, and will keep indefinitely.

---

* From Ehrlich's *Histology of the Blood.*

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### xix. ESBACH'S PICRIC ACID SOLUTION.

| | |
|---|---|
| Picric acid | 10 grammes. |
| Dry citric acid | 20 ,, |
| Distilled water | 1000 c.c. |

### xx. FARRANT'S MEDIUM.

| | |
|---|---|
| Picked gum arabic | 4 ounces. |
| Water | 4 ,, |
| Glycerine | 2 ,, |

### xxi. FEHLING'S REAGENT.

| | | |
|---|---|---|
| A. | Crystallised copper sulphate | 34·64 grammes. |
| | Distilled water up to | 500 c.c. |
| B. | Potassium hydrate | 125 grammes. |
| | Sodium-potassium tartrate | 173 ,, |
| | Distilled water up to | 500 c.c. |

### xxii. FIXATIVE SOLUTION.

Egg albumen is perhaps the best fixative to use.

### xxiii. FORMALIN SOLUTION FOR MUSEUM SPECIMENS.

| | |
|---|---|
| Sodium sulphate | 40 grammes. |
| Magnesium sulphate | 40 ,, |
| Sodium chloride | 20 ,, |

Dissolve in 2000 c.c. of hot water,
add 160 c.c. of formalin.

### xxiv. FORMOL-MÜLLER SOLUTION.

| | |
|---|---|
| Müller's fluid | 98 c.c. |
| Formalin | 2 ,, |

### xxv. GELATINE MEDIUM.

To 1 litre of broth add 100 grammes of gelatine, heat in water till the gelatine is dissolved, neutralise carefully; add white of egg, heat in steriliser till it becomes clear, then filter. Sterilise for two successive days for ½ hour. Be careful not to heat gelatine too long, as it will not set.

### xxvi. GLACIAL ACETIC ACID SOLUTION.

| | |
|---|---|
| Glacial acetic acid ... ... | 1 part by volume. |
| Distilled water ... ... | 300 parts ,, ,, |

### xxvii. GLUCOSE BROTH MEDIA.

To every 100 c.c. of broth media, which should have an alkalinity to litmus (about 5 to 7 c.c. of normal alkali per litre), add 2 grammes of glucose.

### xxviii. GLYCERINE SOLUTION FOR PRESERVING MUSEUM SPECIMENS.

| | |
|---|---|
| Pure glycerine (best) ... | 60 parts by volume. |
| Distilled water ... ... | 40 ,, ,, ,, |
| Sat. sol. of potassium acetate | 2 ,, ,, ,, |
| Formalin ... ... ... | 2 ,, ,, ,, |

Filter before use.

### xxix. GRAM'S IODINE.

| | |
|---|---|
| Iodine ... ... ... | 1 gramme. |
| Potassium iodide ... ... | 2 grammes. |
| Distilled water ... ... | 300 c.c. |

### xxx. GUM ARABIC SOLUTION.

Saturated solution of gum arabic in distilled water. Place an excess of pure gum arabic in distilled water, and keep for 3 days in incubator, shaking occasionally. Filter through fine muslin.

### xxxi. HÆMATEIN.

| | |
|---|---|
| Hæmatein | 1 gramme. |
| Alcohol | 50 c.c. |
| Alum | 50 ,, |

Dissolve by standing in a warm incubator, and add 1000 c.c. of distilled water.

### xxxii. HYPOBROMITE SODIUM SOLUTION.

| | |
|---|---|
| Caustic soda | 100 grammes. |
| Water | 250 c.c. |

Allow mixture to cool, then add

| | |
|---|---|
| Bromine | 25 c.c. |

### xxxiii. JENNER'S STAIN.

*Procure* (1) a bottle of absolute methylic alcohol (that sold by E. Merck for analysis).
(2) Grubler's eosine yellow shade, soluble in water.
(3) Grubler's medicinal methylene blue.
(4) Two perfectly clean, dry, well-stoppered bottles.

In one bottle make a 0·5% solution of the eosine in methyllic alcohol.

In the other bottle make a 0·5% solution of the methylene blue in methylic alcohol.

Keep these tightly stoppered.

For use—

| | |
|---|---|
| Eosine solution | 12·5 c.c. |
| Methylene blue solution | 10 ,, |

Mix them in a perfectly dry, clean, well-stoppered bottle.

*The reagents must be perfectly pure, and the bottles and measuring glass chemically clean, otherwise the stain will not be good.*

*A reliable stain may be obtained from Mr. R. Kanthack*, 18 *Berners Street, W.*

### xxxiv. Litmus Solution (Neutral).

Soak litmus crystals in absolute alcohol for 24 hours, pour off alcohol, and wash with fresh absolute alcohol until the red-green colour has disappeared. Then soak the crystals in distilled water. Decant, and add dilute sulphuric acid to the solution until it is distinctly acid. Now add barium oxide with great care until the solution becomes blue again. Then pass $CO_2$ through the solution until it is again acid. Keep this for stock.

Before use boil a small quantity of the stock solution to drive off the $CO_2$, and the liquid becomes neutral litmus.

### xxxv. Löffler's Methylene Blue.

| | |
|---|---|
| 1 in 1000 solution of caustic potash ... | 100 c.c. |
| Saturated alcoholic sol. of methylene blue ... | 33 ,, |

### xxxvi. Löffler's Serum Medium.

Collect blood, separate the serum. To each litre add 30 c.c. 1% broth, 30 c.c. of glucose, and 5 c.c. of normal caustic soda. Fill into tubes, heat to 60° for ½ hour, on three successive days.

### xxxvii. Marchi's Fluid.

| | |
|---|---|
| Müller's fluid ... ... | 2 parts by volume. |
| Osmic acid, 1% solution ... | 1 part ,, ,, |

### xxxviii. Methyl Violet.

Saturated alcoholic solution of methyl violet.

Distilled water.

Add the methyl violet solution to distilled water till it acquires a fairly deep violet tinge.

### xxxix. Müller's Fluid.

| | |
|---|---|
| Potassium bichromate .. ... | 2·5 grammes. |
| Sodium sulphate ... ... ... | 1 gramme. |
| Tap water ... ... ... ... | 100 c.c. |

Dissolve in hot water.

### xl. Neisser's Stain for Diphtheria Bacilli.

One gramme of methylene blue (Grübler's) is dissolved in 20 c.c. of 96% alcohol, and mixed with 950 c.c. distilled water and 50 c.c. of glacial acetic acid.

*Contrast stain.*—2 grammes of vesuvin are dissolved in 1000 c.c. of boiling distilled water, and the solution filtered after standing.

### xli. Nissl's Methylene Blue Solution.

| | | |
|---|---|---|
| Methylene blue (Methylenblau pat.) ... | 3·75 | parts by weight. |
| Venice soap ... ... | 1·75 | „ „ |
| Distilled water ... | 1000 | „ by measure. |

### xlii. Orcein Stain.

| | | |
|---|---|---|
| Grübler's Orcein ... | 1 | part by weight. |
| Hydrochloric acid ... | 1 | „ by measure. |
| Absolute alcohol ... | 100 | parts „ |

### xliii. Oxalic Acid Solution.

| | | |
|---|---|---|
| Oxalic acid ... ... | 2 | parts by weight. |
| Distilled water... ... | 100 | „ measure. |

### xliv. Potassium Permanganate Solution.

| | |
|---|---|
| Potassium permanganate | 1 part by weight. |
| Water ... ... ... | 400 parts by measure. |

### xlv. Pyrogallic Acid Solution.

| | |
|---|---|
| Pyrogallic acid | 5 parts by weight. |
| Distilled water | 100 ,, ,, measure. |

### xlvi. Soudan III. Solution.

A saturated solution of Soudan III. in 96% spirit Filter.

To this add two-thirds its bulk of 50% spirit, and again filter.

### xlvii. Sodium Tungstate Solution.

| | | |
|---|---|---|
| 1. | Sodium tungstate | 30 parts by weight. |
| | Water | 200 ,, ,, measure. |
| 2. | Sodium tungstate | 30 parts by weight. |
| | 30% solution of acetic acid | 75 ,, ,, measure. |
| | Water | 120 ,, ,, ,, |

### xlviii. Toison's Fluid.

| | |
|---|---|
| Methyl violet | 0·025 grammes. |
| Neutral glycerine | 30·0 c.c. |
| Distilled water | 80 ,, |

Add to this a solution of

| | |
|---|---|
| Sodium chloride | 1 gramme. |
| Sodium sulphate | 8 grammes. |
| Distilled water | 80 c.c. |

Filter the mixture.

### xlix. Van Gieson's Stain.

Saturated solution of picric acid; add a

0·5% solution of acid fuchsine till mixture is a deep red colour.

### l. Von Ebner's Decalcifying Solution.

| | |
|---|---|
| Sodium chloride | 2·5 grammes. |
| Hydrochloric acid | 2·5 c.c. |
| Alcohol | 500 ,, |
| Distilled water | 100 ,, |

### li. Weigert's Differentiating Solution.

| | |
|---|---|
| Borax | 2 grammes. |
| Potassium ferridcyanide | 2·5 ,, |
| Distilled water | 100 c.c. |

### lii. Weigert's Hæmatoxylin.

*Solution I.*

| | |
|---|---|
| Hæmatoxylin | 5 grammes. |
| Methylated spirit | 50 c.c. |

*Solution II.*

| | |
|---|---|
| Lithium carbonate | 1% solution in water. |

| | |
|---|---|
| Solution I. | 5 c.c. |
| Solution II. | 50 ,, |
| Distilled water | 450 ,, |

### liii. Weigert's Iodine.

| | |
|---|---|
| Iodine | 1 gramme. |
| Potassium iodide | 2 grammes. |
| Distilled water | 100 c.c. |

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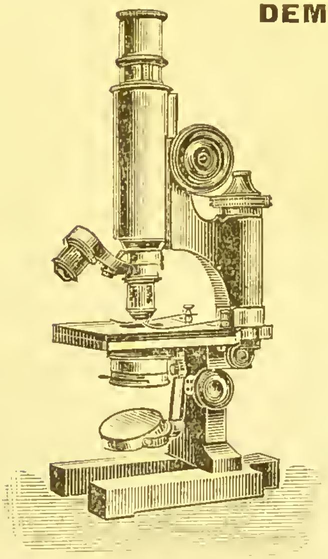

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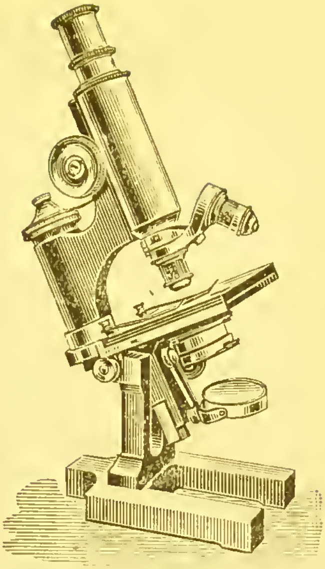

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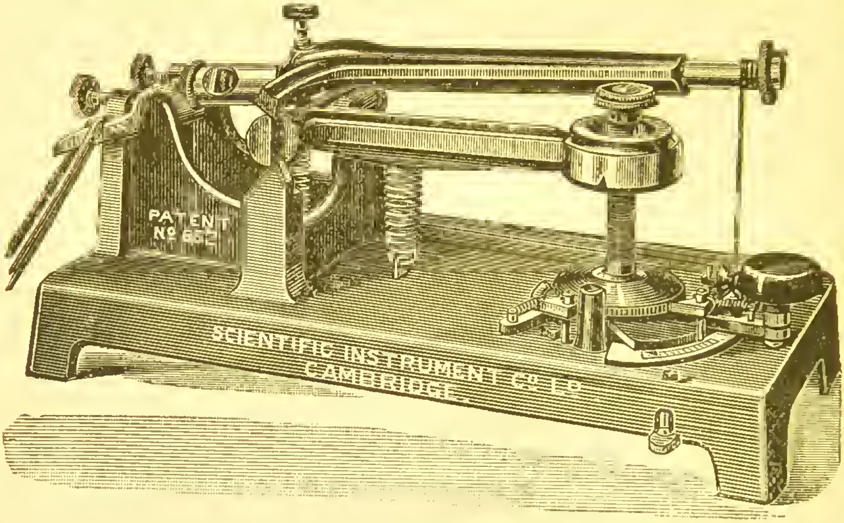

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